Plasma Glucose Turnover in Humans as Studied with C^{14} Glucose

Influence of Insulin and Tolbutamide

G. L. Searle, Ph.D., G. E. Mortimore, M.D., R. E. Buckley, M.D., W. A. Reilly, M.D.,
San Francisco

Much evidence has been accumulated showing that insulin either from functioning pancreatic islet cells, or from continued insulin administration, is requisite for the effective action of the oral hypoglycemic drug, tolbutamide.[1-4] Further, it has been suggested that this agent potentiates the secretion of insulin,[5,6] and it has been reported that both insulin and tolbutamide suppress the output of glucose from the liver.[7-9] In spite of this evidence, the theory that tolbutamide exerts its action through potentiation of insulin secretion has been in serious doubt because of the many negative reports concerning a peripheral action for the drug.[10-12] Further, dissimilarities in intermediary metabolism following the administration of insulin and tolbutamide have been documented.[13]

In order to test the similarity of action of these two compounds on the supply of glucose to the circulation and in an effort to describe further the action of these two drugs on the kinetics of plasma glucose, we have studied both the immediate and prolonged effect of insulin and tolbutamide on the turnover of plasma glucose.

METHODS AND MATERIALS

One hundred microcuries of evenly labeled C^{14} glucose were heat sterilized in 0.9 per cent NaCl solution at a concentration of 0.033 moles/liter.

Blood samples were obtained through indwelling Cournand or Rochester plastic needles in heparinized syringes. Plasma was separated immediately from the whole blood by centrifugation. The plasma proteins were precipitated according to the method of Nelson and Somogyi,[14] and the plasma glucose concentration was determined from aliquots of the plasma protein free filtrate by the method of Folin and Wu.[15] Glucose was isolated from the plasma protein free filtrate as the glucose osazone according to a method described previously.[10] The glucose osazone was assayed colorimetrically in alcohol solution, and an aliquot of this solution was precipitated in water on an aluminum planchet, dried and counted with an end-window Geiger counter. Appropriate mass corrections were made on each sample.

Selection of subjects. Subjects were selected from the medical and orthopedic wards of the hospital on the basis of normal liver function and normal kidney function. The nondiabetic group had no history of diabetes, and the glucose tolerance was normal.

Diabetic subjects were selected on the basis of diabetic history and abnormal glucose tolerance tests. Blood sugar levels of these diabetic subjects could be adequately controlled with diet. None of the diabetic subjects required insulin to prevent the appearance of ketoacidosis.

Experimental design. All subjects were studied first with insulin and then, after a three- to five-week interval, with tolbutamide. Experiments were begun at 9:00 a.m. and concluded by 3:00 p.m. of the same day. Subjects received a light meal at 9:00 p.m. the day preceding the experiment in order to maintain the nutritional state as constant as possible. No food was allowed during these experiments.

At zero time the subject received intravenously 90 μc of sterile C^{14} glucose. Following this, at least three blood samples were withdrawn at approximately half-hour intervals. These samples were immediately centrifuged, and the concentration and specific activity (counts per minute/milligram) of the plasma glucose were determined to establish a predrug turnover curve. Insulin (0.05 units per kilogram intravenously and 0.15 units per kilogram subcutaneously) or tolbutamide (0.025 gm. per kilogram intravenously and 0.065 gm. per kilogram orally) was administered as the test drug. These doses were found to bring about approximately the same reduction of plasma glucose. The dual routes of administration were selected to achieve a rapid initial fall in the plasma glucose as well as maintenance of reduced plasma glucose concentrations for protracted

Presented at the Forty-ninth Annual Meeting of the American Society of Biological Chemists in Philadelphia on April 18, 1958.

From the Radioisotope and Medical Services, Veterans Administration Hospital, San Francisco, California.

periods of time. Blood samples were withdrawn at eight- to fifteen-minute intervals for the first hour following drug administration, then at forty-five- to sixty-minute intervals until the experiments were terminated. These samples were immediately centrifuged, and the concentration and s.a. of the plasma glucose were determined in order to estimate the effects of drug administration on the entry and removal rates of plasma glucose.

RESULTS

The time course of the concentration and s.a. of plasma glucose for three of the subjects studied is shown in figures 1-3 and 1a-3a. Figures 1-3 present the data obtained in the insulin studies while figures 1a-3a present the data obtained in the tolbutamide studies. The simultaneous and equal steady state rates of entry and removal of plasma glucose (plasma glucose turnover), and the unequal nonsteady state rates of entry and removal of plasma glucose have been calculated for each of the fourteen studies reported here. Standard equations for first order reaction kinetics[17] were employed when a constant level of plasma glucose and a logarithmic decline of the s.a.-time curve of plasma glucose were evidenced. When these criteria were not met, nonsteady state calculations as outlined by Dunn and associates,[18] and Jacobs and associates[19] were employed. The results of these calculations for the insulin studies are in table 1, and for the tolbutamide studies in table 2. The calculations are listed as applied to three phases of the studies. These phases were arbitrarily chosen for purposes of discussion and are outlined as follows: Phase 1 is the period following C^{14} glucose administration but prior to drug administration. Phase 2 begins at the time of drug administration and continues to the time the maximal acute depression in plasma glucose is observed. Phase 3 is a time period averaging two hours at the end of the studies after the acute phase of plasma glucose depression when steady state conditions are again evident.

The effect of insulin on the turnover of plasma glucose in nondiabetic and diabetic human subjects. Figures 1 and 3 illustrate a response to insulin characterized by an immediate change in the slope of the s.a.-time curve of plasma glucose to a zero value, indicating a cessation in the supply of nonlabeled glucose to the circulation. The average time duration of this zero slope, noted in four of seven subjects studied with insulin, was approximately thirty minutes. A calculation was made of the rate of removal of glucose from the circulation during this time (Phase 2). The nondiabetic

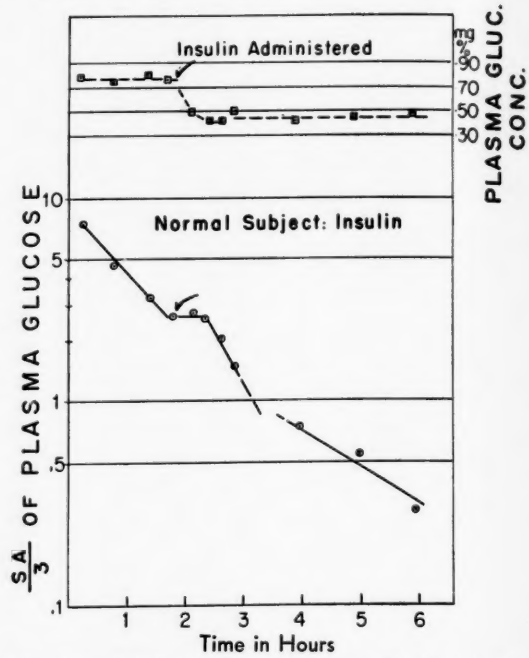


FIG. 1. Nondiabetic (normal) subject A.Z. Weight 41 kg. Insulin (two units intravenously, 4 units subcutaneously) administered at the arrow.

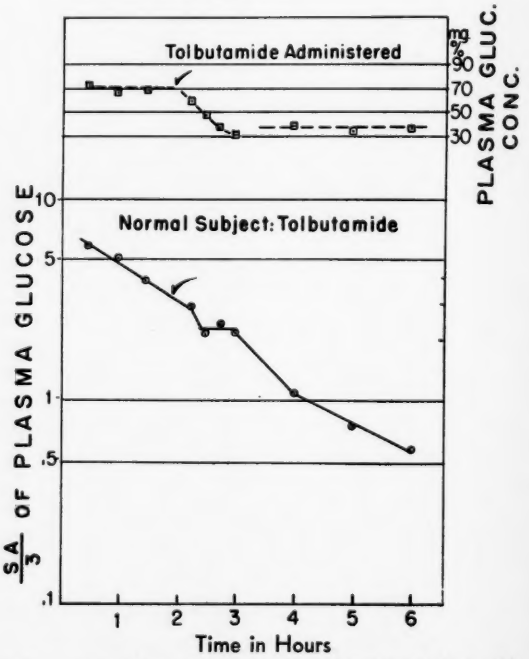


FIG. 1a. Nondiabetic (normal) subject A.Z. Weight 41 kg. Tolbutamide (1.0 gm. intravenously, 2.5 gm. orally) administered at the arrow.

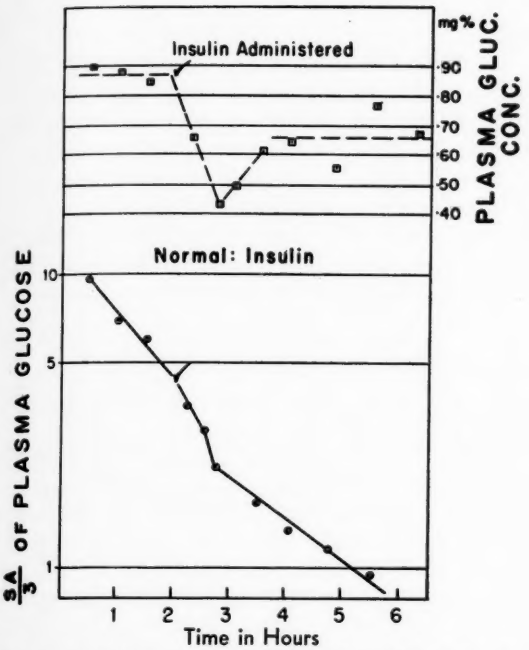


FIG. 2. Nondiabetic (normal) subject D.T. Weight 66 kg. Insulin (3.3 units intravenously, 10.0 units subcutaneously) administered at the arrow.

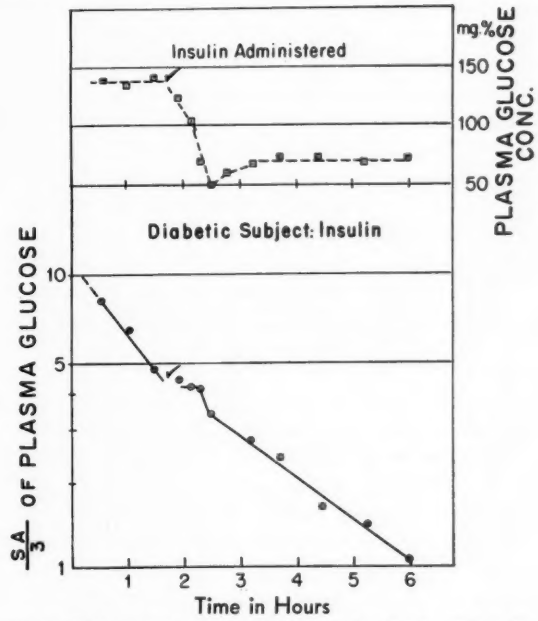


FIG. 3. Diabetic subject F.D. Weight 71 kg. Insulin (3.5 units intravenously, 11.0 units subcutaneously) administered at the arrow.

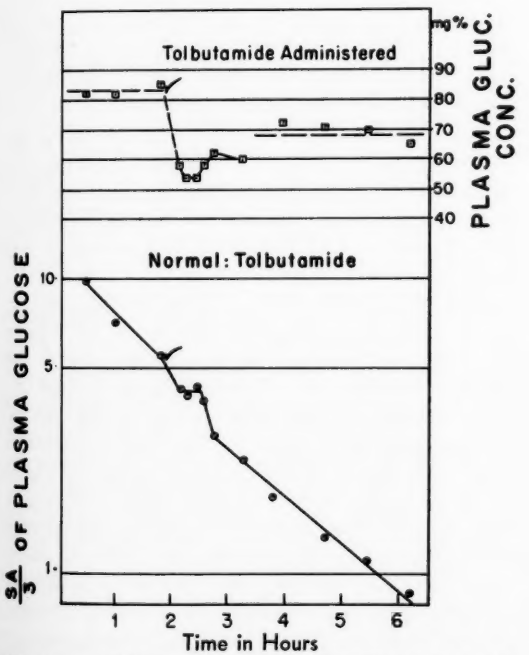


FIG. 2a. Nondiabetic (normal) subject D.T. Weight 66 kg. Tolbutamide (1.65 gm. intravenously, 4.5 gm. orally) administered at the arrow.

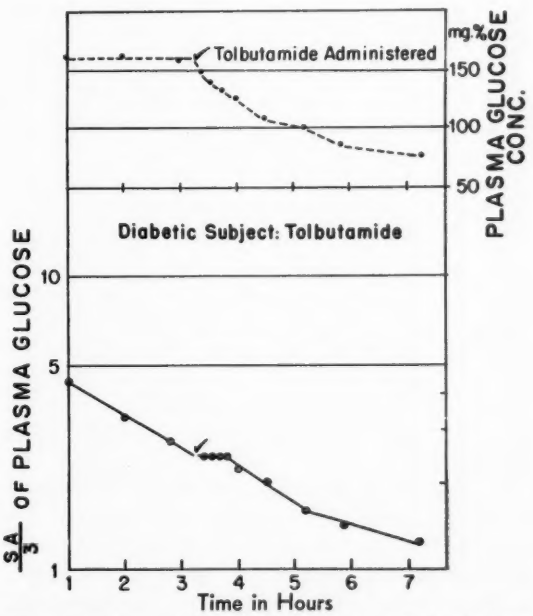


FIG. 3a. Diabetic subject F.D. Weight 71 kg. Tolbutamide (1.77 gm. intravenously, 4.5 gm. orally) administered at the arrow.

TABLE 1

Insulin studies

Subject	State	Weight kg.	Phase 1 Gluc. conc. mg. per cent	Phase 1 T.R.* mg./kg./hr.	Phase 2 E.R.† mg./kg./hr.	Phase 2 R.R.‡ mg./kg./hr.	Phase 3 Gluc. conc. mg. per cent	Phase 3 T.R. mg./kg./hr.
F.N.	Nondiabetic	70	79	108	67	327	37	42
J.J.	"	69	96	91	86	260	48	61
A.Z.	"	41	77	146	0	244	46	56
D.T.	"	66	88	114	111	329	67	54
J.F.	"	72	68	106	0	260	57	42
F.D.	Diabetic	71	139	206	0	294	72	64
C.E.	"	59	95	144	0	163	49	42

*T.R.: Turnover rate—the simultaneous and equal rates of entry and removal of plasma glucose.
†E.R.: Nonsteady state entry rate of plasma glucose.
‡R.R.: Nonsteady state removal rate of plasma glucose.

TABLE 2

Tolbutamide studies

Subject	State	Weight kg.	Phase 1 Gluc. conc. mg. per cent	Phase 1 T.R. mg./kg./hr.	Phase 2 E.R. mg./kg./hr.	Phase 2 R.R. mg./kg./hr.	Phase 3 Gluc. conc. mg. per cent	Phase 3 T.R. mg./kg./hr.
F.N.	Nondiabetic	70	89	141	0	179	55	25
J.J.	"	69	99	122	0	148	77	48
A.Z.	"	41	70	69	0	110	36	31
D.T.	"	66	85	105	0	184	70	64
J.F.	"	72	66	73	0	115	45	69
F.D.	Diabetic	71	162	104	0	107	77	24
C.E.	"	59	162	116	0	112	80	38

subjects studied showed a 151 per cent increase in the rate of removal of plasma glucose following insulin administration. On the other hand the results from the diabetic subjects showed an increase averaging 31 per cent of the pre-insulin value. After the period of zero slope the s.a.-time curve of plasma glucose again declined as the concentration of plasma glucose rose slightly and then stabilized at a value approximately 50 per cent of that observed during Phase 1 in both the nondiabetic and diabetic subjects. When the glucose concentration was again relatively stable and the s.a.-time curve of plasma glucose evidenced a logarithmic decline (Phase 3), a second calculation of the turnover rate of plasma glucose was made. This calculation showed, in both the nondiabetic and diabetic subjects, that the rate of supply and removal of glucose to and from the circulation was remarkably depressed from the postabsorptive values observed in Phase 1 prior to insulin administration.

Figure 2 illustrates a quantitatively different response of the plasma glucose to insulin administration in a nondiabetic subject. Here immediately following insulin, in Phase 2, the s.a.-time curve of plasma glucose changed its slope, not to a zero value, but to an increasingly negative value. A calculation of the rate of glucose supply made during this time indicated that the rate of entry of glucose to the circulation was reduced slightly or not at all. As was demonstrated in the other insulin studies, the rate of removal of plasma glucose following insulin administration was increased. Thus the increase in the negative value of the slope of the s.a.-time curve of plasma glucose was not due to any increase in the rate of supply of glucose, but was the result of an increase in the rate of removal of plasma glucose. A calculation of the turnover rate of plasma glucose in Phase 3 demonstrated a remarkable reduction in rate as was shown in the other insulin studies.

Effect of tolbutamide on the turnover of plasma glucose in nondiabetic and diabetic human subjects. Figures 1a through 3a illustrate the response observed in the concentration and s.a. of plasma glucose before and after the administration of tolbutamide in one diabetic and two nondiabetic subjects. The insulin data from these same subjects were presented in figures 1-3. It will be noted that in each case the response of the s.a.-time curve of plasma glucose following tolbutamide administration was to change its slope to a zero value. The duration of this zero slope, observed in all the tolbutamide studies, was approximately thirty minutes. The rate of supply of nonlabeled glucose to the circulation during this time was zero. A calculation of the rate of removal of glucose from the plasma, during this

time, showed an increase in the five nondiabetic subjects studied which averaged 44 per cent greater than the rate of turnover determined in Phase 1.

In contrast to the observations in the nondiabetic subjects, the diabetic subjects studied showed no increase in the rate of removal of plasma glucose following tolbutamide administration.

A calculation of the turnover rates of plasma glucose made in Phase 3 of the tolbutamide studies showed a reduction in rate, as evidenced in the insulin studies, for both the nondiabetic and diabetic subjects.

DISCUSSION

In the nondiabetic subjects studied here the effects of insulin and tolbutamide were qualitatively similar with respect to changes in the rates of supply and removal of plasma glucose. Insulin or tolbutamide administered in amounts which brought about an approximately equal reduction in the concentration of plasma glucose caused:

1. A transient complete inhibition of the release of glucose into the plasma, a process which normally occurs at an appreciable rate in the postabsorptive state. This complete inhibition occurred consistently with tolbutamide and was observed in two of the five subjects studied with insulin.

2. An acceleration in the removal of glucose from the plasma. This acceleration was greater with insulin than with tolbutamide. In these studies insulin increased the postabsorptive removal rate of plasma glucose in the nondiabetic subjects by an average of 151 per cent. Tolbutamide increased this rate in the nondiabetic subjects by an average of 44 per cent.

3. The release of glucose into the plasma to occur at a greatly reduced rate, when the concentration of plasma glucose was maintained at a low level by subcutaneously administered insulin or orally administered tolbutamide.

In the diabetic subjects studied here the effects of insulin and tolbutamide on the plasma glucose were similar with regard to the supply of glucose to the plasma. A transient complete inhibition of supply was observed in Phase 2 followed by a greatly reduced rate of supply in Phase 3 as compared to Phase 1.

The important difference between the action of insulin and tolbutamide in the diabetic subjects studied here was that no increase was evidenced in the rate of removal of plasma glucose following tolbutamide administration.

The effect of tolbutamide on the hepatic supply of glucose to the circulation reported here is in agreement with the results of a number of investigations recently reported in the literature.[2,7-9,19] However, that this action of tolbutamide is similar to that of insulin is in some dispute. Ashmore and associates[20] have reported that while tolbutamide depresses the output of glucose from the liver of dogs, insulin increases it. These studies employed C^{14} glucose in a manner similar to the present studies. These observations have been confirmed by Mahler and associates[21] by means of portal arterial sampling of blood glucose in dogs. The effect of insulin on the release of glucose from the liver of humans, however, has been recently reported by Jacobs and associates as being inhibitory. These studies were similar in experimental design to the present study.

Evidence for an acute peripheral action of tolbutamide similar to that of insulin, on the other hand, has not been found. Jacobs and associates[19] reported negative findings in this aspect of tolbutamide action, while the findings in the present study show an increase in the removal rate of plasma glucose following tolbutamide administration in nondiabetic subjects. The significance of this peripheral response following tolbutamide is difficult to resolve, especially in the light of the study by Jacobs. Technical differences between these two studies which might explain the variant results are as follows: (1) Plasma glucose was analyzed in the present study, whereas whole blood glucose was analyzed in the study of Jacobs. (2) The amounts and rates of drug administration differed in the two studies. In connection with the second point, two recent studies[10,22] have shown the rate and route of administration as being important in eliciting a peripheral response with exogenous insulin. Jacobs and associates were unable to obtain an increase in the rate of removal of blood glucose when they administered insulin subcutaneously, however, they did notice an effect on the supply of glucose to the circulation similar to that following tolbutamide administration. Madison and Unger reported that insulin injected intraportally fails to elicit the same response in arteriovenous glucose differences that are seen when insulin is given intravenously. A more recent report by Butterfield and associates[23] demonstrated a threshold for the uptake of glucose by the peripheral tissues. This threshold was reported to be elevated in the diabetic subject and to be lowered by insulin and two of the oral hypoglycemic compounds, tolbutamide and phenethyldiguanide. These results demonstrated a peripheral effect in the diabetic subject after some length of successful treatment with the oral hypoglycemic compounds. An acute effect of tolbutamide, in the nondiabetic subject, has been demonstrated in the current study, however, it was not possible to demonstrate this same effect in the diabetic sub-

ject. This seems reasonable since these same diabetic subjects seemed to demonstrate some degree of peripheral insulin insensitivity in these studies. It will be recalled that the nondiabetic subjects exhibited an increase in the removal rate of plasma glucose following insulin that averaged 151 per cent greater than the pre-insulin rate of turnover of plasma glucose. The diabetic subjects, however, demonstrated an increase in the rate of removal of plasma glucose following insulin of only 31 per cent greater than the pre-insulin rate of turnover of plasma glucose.

Many of the recent reports comparing the action of tolbutamide and insulin have emphasized the easily detectable peripheral action of insulin, i.e., an increase in the arteriovenous glucose difference[22] or an acceleration in the rate of removal of glucose from the circulation.[19] In the present study a similar acceleration in the rate of removal of plasma glucose following insulin was demonstrated. In addition we have shown that the prolonged action of insulin or tolbutamide (sustained hypoglycemia) caused a marked reduction in the rate of turnover of plasma glucose. Since the turnover rate is a measure of the simultaneous and equal rates of entry and removal of glucose to and from the circulation, it can be assumed that the rate of removal is dependent on the rate at which glucose can be supplied to the plasma in order to maintain a constant concentration of plasma glucose. Therefore a prime action of insulin or tolbutamide may be to cause the liver to exert a rate-limiting effect on the over-all metabolism of plasma glucose.

SUMMARY

The kinetics of supply and removal of plasma glucose following the administration of insulin or tolbutamide have been investigated. A comparison of the effects of these two compounds has shown that they act very similarly in lowering the plasma glucose in nondiabetic subjects. Both insulin and tolbutamide caused an inhibition of the supply of plasma glucose and an acceleration in the rate of removal of plasma glucose, immediately following their administration in nondiabetic subjects. With the prolonged action of either drug (maintenance of a depressed level of plasma glucose) it is suggested that the rate of supply of glucose to the plasma is a rate-limiting factor in the utilization of plasma glucose.

In the diabetic subject similar effects of the two drugs were noted on the supply of glucose to the plasma, i.e., an inhibition of supply. However, while a stimulation in the rate of removal of plasma glucose was noted with insulin, this was not the case with tolbutamide.

SUMMARIO IN INTERLINGUA

Le Influentia De Insulina E De Tolbutamido Super Le Metabolismo Plasmatic De Glucosa In Humanos, Studiate Per Medio De Glucosa A C^{14}

Esseva investigate le cinetica que reduce le nivello plasmatic de glucosa post le administration de insulina o tolbutamido. Un comparation del effectos de iste duo compositos ha monstrate que illos age in un maniera multo simile in tanto que illos reduce le glucosa del plasma in subjectos non-diabetic. Tanto insulina como etiam tolbutamido causava un inhibition del provision del plasma con glucosa e un acceleration del remotion de glucosa ab le plasma, immediatemente post lor administration a subjectos non-diabetic. Con le prolongation del action del un o del altere del duo drogas (mantenentia de un deprimite nivello del glucosa de plasma), il pare que un reducite alimentation del plasma con glucosa age como factor limitatori in le utilisation plasmatic de glucosa.

In subjectos diabetic, il esseva notate que le duo drogas produceva simile effectos in le alimentation del plasma con glucosa, i.e. illos inhibiva ille alimentation. Tamen, durante que un intensificate elimination de glucosa ab le plasma esseva notate in le caso de insulina, isto non esseva ver in le caso de tolbutamido.

ACKNOWLEDGMENT

This work was aided by a grant from The Upjohn Company, Kalamazoo, Michigan.

We are indebted to Dr. William R. Kirtley of Eli Lilly and Company for generous supplies of HGF-free insulin, and to Dr. C. J. O'Donovan of The Upjohn Company for generous supplies of tolbutamide (Orinase).

REFERENCES

[1] Houssay, B. A., Penhos, J. C.: Action of the hypoglycemic sulfonyl compounds in hypophysectomized, adrenalectomized and depancreatized animals. Metabolism 5:727, 1956.

[2] Goetz, F. C., Gilbertsen, A. S., Josephson, V.: Acute effects of Orinase on peripheral glucose utilization. Metabolism 5:788, 1956.

[3] Moorhouse, J. A., Kark, R. M.: Physiologic actions of Orinase and their relationships to types of diabetes in man. Metabolism 5:847, 1956.

[4] Houssay, B. A., Penhos, J. C., Urgotti, E., Teodosio, N., Apelbaum, J., Bowkett, J.: The role of insulin in the action of the hypoglycemic sulfonyl compounds. Ann. N.Y. Acad. Sci. 71:(1):25, 1956.

[5] Volk, B. W., Goldner, M. G., Weisenfeld, S., Lazarus, S. S.: Functional and histological studies concerning the action of the sulfonylureas. Ann. N.Y. Acad. Sci. 71(1):141, 1957

[6] Colwell, A. R., Colwell, J. A., Colwell, A. R., Sr.: Intrapancreatic perfusion of the antidiabetic sulfonylureas. Metabolism 5:749, 1956.

[7] Anderson, G. E., Perfetto, A. J., Termine, C. M., Monaco, R. R.: Hypoglycemic action of Orinase, effect on output of glucose by liver. Proc. Soc. Exp. Biol. & Med. *92*:340, 1956.

[8] Tyberghein, J. M., Halsey, Y. D., Williams, R. H.: Action of butyltolylsulfonylureas on liver glycogenolysis. Proc. Soc. Exp. Biol. & Med. *92*:322, 1956.

[9] Schambye, P., and Tarding, F.: The action of hypoglycemic sulfonylureas upon the blood sugar regulation. Acta Physiol. Scand. *42*, 145: 124, 1957.

[10] Renold, Albert E., Winegrad, A. L., Froesch, E. R., and Thorn, G. W.: Studies on the site of action of the arylsulfonylureas in man. Metabolism *5*:757, 1956.

[11] Wick, A. N., Britton, B., and Grabowski, R.: The action of a sulfonylurea hypoglycemic agent (Orinase) in extrahepatic tissues. Metabolism *5*:739, 1956.

[12] Elrick, H., and Purnell, R.: The response of kidney, liver and peripheral tissues to tolbutamide and insulin. Ann. N.Y. Acad. Sci. *71*:Art.1, 38, 1957.

[13] Hennes, A. R., Wajchenberg, B. L., Fajans, S. S., Conn, J. W.: Comparative effects of insulin and Orinase on blood levels of pyruvate and alphaketoglutarate in normal subjects. Metabolism *6*:63, 1957.

[14] Nelson, N.: A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. *153*: 375, 1944.

[15] Folin, O., Wu, H.: A simplified and improved method for determination of sugar. J. Biol. Chem. *41*:367, 1920.

[16] Searle, G. L., and Chaikoff, I. L.: Inhibitory action of hyperglycemia on delivery of glucose to the blood stream by the liver of the normal dog. Am. J. Physiol. *170*:456, 1952.

[17] Zilversmit, D. B., Entenman, C., and Fishler, M. C.: On the calculation of "turnover time" and "turnover rate" from experiments involving the use of labeling agents. J. Gen. Physiol. *26*:325, 1943.

[18] Dunn, D. F., Friedmann, B., Maass, A. R., Reichard, G. A., and Weinhouse, S.: Effects of insulin on blood glucose entry and removal rates in normal dogs. J. Biol. Chem. *225*(1): 225, 1957.

[19] Jacobs, G., Reichard, G. A., Goodman, E. H., Friedman, B., and Weinhouse, S.: Action of insulin and tolbutamide on blood glucose entry and removal. Diabetes *7*:358, 1958.

[20] Ashmore, J., Cahill, G. F., Jr., Earle, A. S., and Zottu, S.: Studies on the disposition of blood glucose. Diabetes *1*:1, 1958.

[21] Mahler, R., Shoemaker, W. C., and Pugh, D. E.: The effect of insulin on hepatic glucose production in normal dogs. Program of the Fortieth Meeting, The Endocrine Society, June 1958.

[22] Madison, L. L., and Unger, R. H.: The physiologic significance of the secretion of endogenous insulin into the portal circulation. J. Clin. Investigation *37*:631, 1958.

[23] Butterfield, J., Fry, I. K., and Holling, E.: Effects of insulin, tolbutamide, and phenethyldiguanide on peripheral glucose uptake in man. Diabetes *7*:449, 1958.

Sidelights on Longevity

Impressive strides in increasing the average number of remaining years of life are far-reaching:

— If a child under eighteen is orphaned today, chances are twice as great that he will lose his father instead of his mother; 2 per cent of the orphans in the United States have lost both parents; 29 per cent have lost their mothers only, and 69 per cent have lost their fathers.

— There are fewer chances today, however, of a child under eighteen becoming an orphan. As late as 1920, 16 per cent of our children had lost one or both of their parents due to death. With the marked gains in saving lives in the middle and older ages, this figure has now been reduced to 5 per cent.

— Although there are now almost ten million widowed persons (three quarters of them women) in this country, the proportion of *young* widows and widowers is going down. In 1900, H.I.F. reported, widowed persons under the age of forty-five constituted nearly one-quarter of all the widowed; by 1957 the proportion had dropped to about 6 per cent.

— Men have also benefited from reduced mortality as shown by an increase in the average length of working life expectancy. Males born in 1900 had an average working life expectancy of thirty-two years. Today this figure has increased to forty-two years.

— Gains in average length of life in the United States have steadily increased the proportion of our population in the older ages. In 1900, only 4 per cent of the population was in the age group sixty-five and over; in 1958 that figure was 9 per cent. In fifty-eight years our population over sixty-five has increased by twelve million.

From *Progress in Health Services,* September, 1958, and other issues, Health Information Foundation.

Vascular Disease in Diabetes Mellitus

The Relation of Raised Blood Protein-bound Hexose Levels

I. Kelsey Fry, M.R.C.P., J. R. Trounce, M.D., and C. A. G. Cook, F.R.C.S., with the technical assistance of T. R. Nichols, London

Glycoproteins are proteins to which an appreciable amount of carbohydrate is firmly bound.[1] The blood level of these protein-polysaccharide complexes in diabetes has been extensively studied and many workers have found high levels, particularly in patients with vascular complications.[2-6]

This observation is important because a substance with the staining properties of glycoproteins is intimately related to the histological lesions in the diabetic kidney and retina.[7,8]

Consequently, it has been suggested that high blood levels may lead to the deposition of glycoproteins in the renal and retinal tissues and that this mechanism may be responsible, in part, for the development of the specific "capillary" lesions of diabetes.[7,8]

Because of the parallel biochemical and histological findings, most of the studies of glycoproteins in diabetes have been directed toward the correlation between abnormal levels in the blood and evidence of retinal and renal disease, particularly the Kimmelstiel-Wilson syndrome.

The interpretation of these studies is difficult because many patients have more than one type of vascular disease, so that those with "capillary" complications frequently show evidence also of hypertension or disease of the peripheral vessels. In our opinion insufficient attention has been paid to this association of different types of vascular disease when considering the significance of raised glycoprotein levels in the pathogenesis of diabetic vascular disease, especially in view of reports of raised glycoprotein levels in hypertension,[9,10] peripheral vascular disease,[11] and advanced renal disease,[2] in patients without diabetes.

In the present study we have correlated the serum levels of glycoprotein, estimated as protein-bound hexose, with each of the main types of diabetic vascular disease, in an attempt to separate the contribution of each type to the high levels found by previous workers.

From Guy's Hospital, London, S.E. 1, England.

CLINICAL MATERIAL AND METHODS

We studied fifty-nine patients attending the diabetes clinic at Guy's Hospital. Each was given a thorough examination with the emphasis on the early signs of vascular disease. A fundus examination was carried out by an ophthalmologist (C.A.G.C.) in those patients on whom a recent specialist's report was not available. All patients had a chest X-ray and blood count and none was included who had clinical evidence of any concurrent condition known to be associated with abnormal blood glycoprotein levels such as infection, neoplasia or recent cardiac infarction.[12]

We divided the vascular complications of diabetes into four groups: arterial disease, hypertension, diabetic retinopathy and renal disease. We did not consider patients with diabetic neuropathies as a separate group because numerous clinical entities are included under this heading and the relation of many of them to vascular disease is still obscure.

We diagnosed *arterial disease* if there was a history of angina pectoris or intermittent claudication, if one or more of the peripheral pulses was impalpable or if the retinal arteries showed evidence of degenerative changes in the form of irregularity of caliber, arteriovenous "nipping" and an increased light reflex. A diastolic pressure of 100 mm. Hg or more was taken to represent *hypertension.*

Renal disease was diagnosed if albumin was found in the urine in a concentration of more than 0.2 parts per 1,000.

Diabetic retinopathy was considered to be present if any micro-aneurysms were seen.

Thirty-five of the patients were female and twenty-four male; the youngest was twenty-two and the oldest seventy. The duration of diabetes ranged from six months to thirty-one years. Fifteen patients had no signs of complications; of the remaining forty-four, nineteen had more than one type of vascular disease. Of the twenty-five in whom there was evidence of only one complica-

tion, five had retinopathy, five had albuminuria, nine had hypertension and six had arterial disease. No patient had an overt urinary tract infection; only one had retinitis proliferans.

Nineteen healthy ambulant subjects with normal blood pressure provided control data; their ages ranged from twenty-two to sixty-nine years.

Estimation of protein-bound hexose. Samples of venous blood were taken during routine visits to the diabetes clinic, usually one or two hours after lunch. Protein-bound hexose was estimated by the method described by Winzler.[12] The results are expressed as milligrams hexose per 100 ml. serum.

In nineteen patients further samples were taken at a subsequent visit, at least four weeks after the first. The mean difference between the values obtained on two separate occasions in each patient was 4.4 mg./100 ml. (S.E.M. 0.93).

RESULTS

The mean value for protein-bound hexose in the group of control subjects was 122.4 mg./100 ml. This agrees closely with the values obtained by Andreani and Gray[4] and Winzler.[12]

Table 1 shows the relation between the presence of hypertension and arterial disease and the blood glycoprotein levels. Part A of the table shows the results obtained in the whole group of fifty-nine diabetics, including twenty-eight who also had evidence of diabetic retinopathy or albuminuria. In the absence of arterial disease or hypertension there was no significant difference between the levels of protein-bound hexose in the diabetics and in the control subjects. In the patients who had hypertension without arterial disease the level was significantly higher ($p = <0.001$); a high level was also found in those who had arterial disease without hypertension ($p = <0.001$). When all patients with evidence of these two complications either alone or in combination were considered together the mean value for the whole group was 137.7 mg./100 ml. compared with a mean value of 124.1 mg./100 ml. for those without these complications ($p = <0.001$).

Because of the possibility that coincident diabetic retinopathy or albuminuria might have been contributing to these high values of protein-bound hexose, we analyzed the figures again after excluding the twenty-eight patients with either of these conditions. Part B of table 1 shows the mean values obtained. It can be seen that the mean value of protein-bound hexose for each group was similar to that obtained before. Further, although the number of patients in each group was now smaller, the differences between the values obtained in patients with hypertension or arterial disease compared with those having no such complications were statistically significant (for the hypertensive group $p = <0.001$; for the group with arterial disease alone $p = <0.01$).

These findings are illustrated in figure 1 where the individual values obtained in patients with arterial dis-

TABLE 1

Mean values of protein-bound hexose in patients with and without evidence of hypertension or arterial disease

A. Values for the whole group of patients studied including those with diabetic retinopathy or albuminuria

	Normal controls	Diabetics			
		Hypertension 0 Arterial dis. 0	Hypertension + Arterial dis. 0	Arterial dis. + Hypertension 0	Arterial dis. + and/or Hypertension +
Number of patients	19	25	10	11	34
Average age (yrs.)	44.8	43.6	58.8	57.1	57.2
Protein-bound hexose mg./100 ml. (S.E.M.)	122.4 (2.1)	124.1 (1.5)	138.6 (3.7)	136.8 (3.5)	137.7 (2.3)

B. Values for those patients who showed no evidence of diabetic retinopathy or albuminuria

	Normal controls	Diabetics			
		Hypertension 0 Arterial dis. 0	Hypertension + Arterial dis. 0	Arterial dis. + Hypertension 0	Arterial dis. + and/or Hypertension +
Number of patients	19	15	9	6	16
Average age (yrs.)	44.8	40.2	59.2	54.3	58.8
Protein-bound hexose mg./100 ml. (S.E.M.)	122.4 (2.1)	124.5 (2.3)	140.7 (3.5)	141.0 (5.6)	139.4 (2.9)

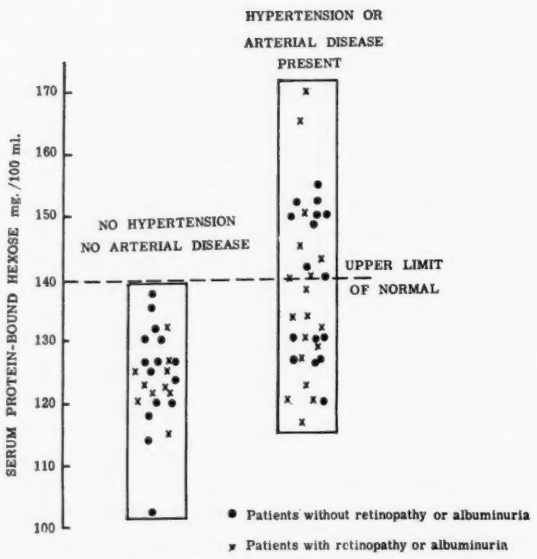


FIG. 1. Individual values of protein-bound hexose in diabetic patients with and without evidence of arterial disease or hypertension. The upper limit of normal is represented by a value two standard deviations above the mean level for nondiabetic control subjects.

ease or hypertension are compared with those in patients with no evidence of these complications. It can be seen that in the absence of hypertension or arterial disease no patient had abnormally high levels of protein-bound hexose, taking as the upper limit of normal a value two standard deviations above the mean. Of the patients in whom these complications were found, some had abnormally high values but many were within normal limits. The figure also indicates those patients in whom there was retinopathy or albuminuria; the values from these patients can be seen scattered evenly through each group.

On this evidence, it would appear that the higher levels of protein-bound hexose were found in association with arterial disease and hypertension and not with diabetic retinal or renal disease. Study of the values obtained in patients with diabetic retinopathy or albuminuria confirmed these findings. Table 2 compares the mean values from patients who had these complications with those from patients in whom they were absent. In part A of the table the values obtained in the whole group of fifty-nine diabetics are shown, including thirty-four with evidence also of hypertension or arterial disease. It can be seen that the mean value for diabetics with no retinopathy or albuminuria was higher than that for controls and that it was not changed significantly when patients with albuminuria or retinopathy were considered. That is to say, in a group of patients, some of whom had hypertension or arterial disease, the presence or absence of retinopathy or albuminuria made little difference to the blood level of protein-bound hexose. That the slightly raised values in all the groups of diabetics in part A of table 2 could be explained by the inclusion of patients with hypertension or arterial disease is indicated in part B. This shows the values obtained when patients with hypertension or arterial disease were excluded. It can be seen that all groups, whether in the presence or absence of retinopathy or albuminuria, had mean values which were not significantly different from those of the normal controls.

These findings are illustrated in figure 2, which shows the individual values found in patients with retinopathy or albuminuria and in those with no evidence of these complications. The figure also indicates the patients in whom there were signs of arterial disease or hypertension. It can be seen that a proportion of patients in each group had abnormally high levels of protein-bound hexose and that in both groups the high levels occurred only in those patients with arterial disease or hypertension.

Table 1 shows that hypertension and arterial disease tend to occur in the older diabetics and it is known[13] that blood glycoprotein levels rise with age. It might be thought, therefore, that the higher values found in patients with these complications were only a reflection of the normal aging process. For this reason we compared the values obtained in two groups of patients, matched in pairs for age, one group having either hypertension or arterial disease, the other having neither (table 3). The difference between the two groups was statistically significant ($p = <0.001$). Further, it can be seen that the mean value for serum protein-bound hexose in both groups was similar to that found when patients of all ages were considered, as in table 1. We therefore concluded that age was not an important factor in producing the high levels of protein-bound hexose associated with hypertension and vascular disease in this series of patients.

DISCUSSION

These results suggest to us that it is unlikely that the raised blood glycoprotein levels found in some diabetics are related to the presence of the specific retinal and renal lesions. This differs from the conclusions of most previous workers.[2-5] The discrepancy could be due in part to the separation of each type of vascular disease, and in part to our emphasis on the early stages of the complications; for instance in one young patient, aged thirty-four, with a protein-bound hexose level of 152 mg./100 ml., well marked retinal arteriosclerosis was

TABLE 2

Mean values of protein-bound hexose in patients with and without evidence of diabetic retinopathy and albuminuria

A. Values for the whole group of patients studied, including those with arterial disease or hypertension

	Normal controls	Diabetics			
		Retinopathy 0 Albuminuria 0	Retinopathy + Albuminuria 0	Albuminuria + Retinopathy 0	Retinopathy + and/or Albuminuria +
Number of patients	19	31	13	8	28
Average age (yrs.)	44.8	49.8	62.2	44.1	53.1
Protein-bound hexose mg./100 ml. (S.E.M.)	122.4 (2.1)	132.2 (2.3)	126.0 (2.0)	127.1 (3.0)	131.7 (2.7)

B. Values for those patients who showed no evidence of arterial disease or hypertension

	Normal controls	Diabetics			
		Retinopathy 0 Albuminuria 0	Retinopathy + Albuminuria 0	Albuminuria + Retinopathy 0	Retinopathy + and/or Albuminuria +
Number of patients	19	15	5	5	10
Average age (yrs.)	44.8	40.2	58.8	38.6	48.7
Protein-bound hexose mg./100 ml. (S.E.M.)	122.4 (2.1)	124.5 (2.3)	123.6 (1.2)	123.4 (2.7)	123.5 (1.4)

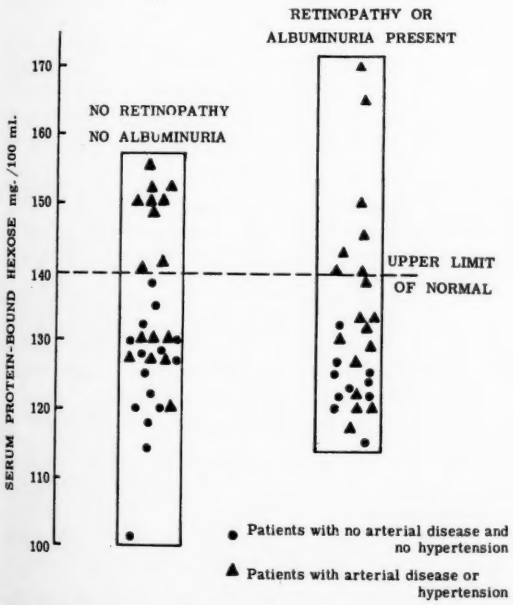


FIG. 2. Individual values of protein-bound hexose in diabetic patients with and without diabetic retinopathy or albuminuria. The upper limit of normal is represented by a value two standard deviations above the mean level for nondiabetic control subjects.

the only evidence of vascular involvement. This emphasis on the earlier recognition of the complications has helped to isolate the relation between each type of

TABLE 3

Mean values for protein-bound hexose in two groups of diabetics, matched in pairs for age, one showing evidence of arterial disease or hypertension, the other without these complications. In these groups, the presence or absence of diabetic retinopathy or albuminuria has not been taken into account.

	No hypertension No arterial disease	Arterial disease and/or hypertension present
Number of patients	14	14
Average age (yrs.)	47.3	47.4
Protein-bound hexose mg./100 ml. (S.E.M.)	124.6 (1.2)	143.1 (3.9)

vascular disease and the blood glycoprotein level, for by the time that there is evidence of advanced retinal and renal disease other vascular complications are likely to be found. In the Kimmelstiel-Wilson syndrome, for example, hypertension is by definition always present, so that at this late stage it is difficult to analyze the effect of the presence of each type of vascular complication on the biochemical findings.

While we found no correlation between raised levels of protein-bound hexose and the "capillary" complications of diabetes, we did obtain evidence that the raised levels could be accounted for by the presence of hypertension and arterial disease, conditions which are known

to be associated with abnormal levels in nondiabetic patients.[9–11] This raises the possibility that high glycoprotein levels in the blood might contribute to the development of hypertension and arterial disease in diabetes. If this were so, one might expect to find raised blood levels before the appearance of clinical evidence of these complications. This was not our experience. Of the fifteen patients without complications whom we investigated, none had levels of protein-bound hexose above normal (figure 1). On the other hand, many patients in whom there were signs of arterial disease or hypertension had normal levels of glycoprotein, particularly in the older age groups.

Although this evidence suggests that the onset of diabetic vascular disease is not related to high serum levels of protein-bound hexose, it should be pointed out that this estimation is an index only of the amount of glycoprotein present and gives no indication as to whether the glycoproteins are normal or abnormal. It remains possible, therefore, that the development of complications is associated with the presence in the blood of abnormal glycoproteins which might be formed as a result of the disordered carbohydrate metabolism.

We wish also to emphasize that, although we have found evidence of an association between high levels of protein-bound hexose and the presence of hypertension and arterial disease, we have no evidence that this is a causal relationship. It may be that the raised levels are associated, for example, with bad control or prolonged duration of the diabetes, factors that are themselves possibly related to the development of vascular disease.

Even so, from the evidence presented here, two conclusions can be drawn. First, that in the patients whom we studied there was no relation between high blood levels of protein-bound hexose and the occurrence of diabetic retinopathy or albuminuria and, second, that when high levels were found they could be explained by the presence of arterial disease or hypertension.

SUMMARY

Blood levels of protein-bound hexose have been related to the presence of vascular complications in fifty-nine diabetics. Evidence of early arterial disease and hypertension was recorded in addition to evidence of the more specific renal and retinal lesions. Fifteen patients with no complications showed values within the normal range. Diabetic retinopathy and albuminuria were not associated with high levels. On the other hand, abnormal values were common in patients with hypertension and arterial disease. The significance of these findings is discussed.

SUMMARIO IN INTERLINGUA

Morbo Vascular In Diabete Mellite: Le Relation De Elevate Nivellos Sanguinee De Hexosa Ligate A Proteina

In cinquanta-nove patientes, determinationes del nivellos sanguinee de hexosa ligate a proteina esseva correlationate al presentia de complicationes vascular. Indicios de precoce morbo arterial e de hypertension esseva registrate a parte le indicios del plus specific lesiones renal e retinal. Dece-cinque patientes con nulle complicationes exhibiva valores intra le limites normal. Retinopathia e albuminuria diabetic non esseva associate con alte nivellos. Del altere latere, valores anormal esseva commun in patientes con hypertension e morbo arterial. Le signification de iste constatationes es discutite.

ACKNOWLEDGMENT

We should like to thank Dr. Christopher Hardwick, Physician to the Diabetic Clinic, Guy's Hospital, for permission to study patients under his care and for his help and encouragement.

REFERENCES

[1] Winzler, R. T.: Chemistry and Biology of Mucopolysaccharides. Ciba Foundation. London, Churchill, 1958, p. 246.

[2] Berkman, J., Rifkin, H., and Ross, G.: Serum polysaccharides in diabetic patients with and without degenerative vascular disease. J. Clin. Invest. *32*:415, 1953.

[3] Adlersberg, D., Wang, C. I., Rifkin, H., Berkman, J., Ross, G., and Weinstein, C.: Serum lipids and polysaccharides in diabetes mellitus. Diabetes *5*:116, 1956.

[4] Andreani, D. V., and Gray, C. H.: Serum polysaccharides in diabetes mellitus. Clin. chim. acta *1*:7, 1956.

[5] Gilliland, I. C., Hanno, M. G., and Strudwick, J. I.: Protein-bound polysaccharides in diabetes with and without complications. Biochem. J. *56*:xxxii, 1954.

[6] Introzzi, P., Bernasconi, C., and Buscarini, L.: Serum proteins, lipids and protein-bound carbohydrates in vascular complications of diabetes mellitus. Acta med. scandinav. *160*:47.

[7] McManus, J. F. A.: The development of intercapillary glomerulosclerosis. Proc. Am. Diabetes A. *9*:303, 1949.

[8] Friedenwald, J. S.: Diabetic retinopathy. Am. J. Ophth. *33*:1187, 1950.

[9] Bastenie, P. A., Verbiest, M., and Conrad, V.: Polysaccharides du serum dans l'hypertension et le diabète. Acta clin. belg. *10*:1, 1955.

[10] Rifkin, H., and Petermann, M. L.: Serum and urinary proteins in diabetic glomerulosclerosis; results of electrophoretic analysis. Diabetes *1*:28, 1952.

[11] Voigt, K. D., and Schrader, E. A.: Untersuchungen über das Verhalten der Lipo- und Glykoproteide bei arteriographisch gesicherten Arteriosklerosen. Klin. Wchnschr. *33*:465, 1955.

[12] Winzler, R. T.: Methods of Biochemical Analysis, ed. Glick, D., Vol. 2, New York, Interscience Publishers, 1955, p. 279.

[13] Shetlar, M. R., Foster, J. V., Kelly, K. H., and Everett, M. R.: Serum polysaccharide level in normal state. Proc. Soc. Exper. Biol. & Med. *69*:507, 1948.

Dietary Linoleic Acid and Linoleate

Effects in Diabetic and Nondiabetic Subjects with and without Vascular Disease

L. W. Kinsell, M.D., G. D. Michaels, Ph.D., G. Walker, M.D., P. Wheeler, Ph.D., S. Splitter, M.D., and P. Flynn, M.D., with the technical assistance of H. P. Chin, M. Coehlo, J. Conklin, G. Fukayama, L. Gammon, F. Olson and S. Smyrl, Oakland

In the present paper, the relationship of dietary linoleic acid to a variety of plasma lipids in diabetic and nondiabetic subjects, for the most part having significant vascular disease, will be considered. The possible relationship of such dietary management to the progress of the vascular disease will also be commented upon.

METHODS

Some studies were carried out in ambulatory patients on semiconstant dietary intake. Some were carried out in patients on the metabolic ward receiving quantitatively constant dietary intake. In the latter group, all food was consumed as formula mixture at hourly intervals from 9 a.m. to 9 p.m. These formula diets contained adequate protein derived from fat-free milk solids or from calcium caseinate, and were fortified with appropriate amounts of essential vitamins and minerals, and 10 gm. of crude liver powder daily to provide adequate amounts of trace materials. Unless otherwise stated, all patients were in nitrogen equilibrium or positive nitrogen balance throughout the period of study.

The methods used for determination of plasma cholesterol, phospholipid and total lipid have been noted elsewhere.[1,7] Plasma lipids were fractionated by column chromatography.[2] The method used for alkaline isomerization and determination of the fatty acids is described elsewhere.[3] In recent studies, fatty acids have been determined by gas-liquid chromatography[4a] as well as by ultraviolet photometry after isomerization.

Some comment may be in order regarding gas-liquid chromatography. The technic was devised by James and Martin.[4b] The fundamental principle is that of qualitative-quantitative separation of a variety of materials on the basis of their boiling points and molecular weights. In the case of the fatty acids, using liquid phase materials such as those devised by Lipsky[4c] one is able to get excellent separation and quantitative recovery of the eighteen carbon fatty acids, stearic, oleic, linoleic, and linolenic, as well as of acids with differing chain length.

Using instruments in which the detection device is a thermal conductivity cell, one can accurately measure as little as 0.1 mg. of material. Using ionization detectors, as little as .002 μg of fatty acid has been measured accurately.

Purified materials for accurate standardization are available for many unbranched, saturated and unsaturated fatty acids up to and including C-20. Beyond this chain length, no standards are available for the unsaturated acids. It is probable that this situation will steadily improve.

EXPERIMENTAL OBJECTIVES

In all of our studies, certain specific objectives have been pursued:

1. Determination of possible specific ratio relationships between dietary unsaturated and saturated fatty acids, with reference to the production of specific levels of plasma cholesterol, phospholipids, and neutral fat.

2. Clarification of the question: Will linoleic acid, administered as highly purified ethyl linoleate, produce the same effect upon plasma lipids that one observes with the administration of neutral fats, which contain abundant linoleic acid?

3. Determination of the effects of purified ethyl oleate upon blood lipids.

Presented at the Eighteenth Annual Meeting of the American Diabetes Association in San Francisco on June 22, 1958.

From the Institute for Metabolic Research, Highland-Alameda County Hospital, Oakland, California.

4. Determination of the qualitative and quantitative effects of dietary fatty acids upon fatty acid composition of specific plasma lipids.

5. Determination of the presence or absence of clinical improvement in existing atherosclerosis, and/or existing diabetic retinal and renal disease during the long-term administration of natural or "synthetic" fats containing large amounts of linoleic acid.

EXPERIMENTAL OBSERVATIONS

Ratio of unsaturated to saturated fat. It is well established that the administration of diets containing 80 to 150 gm. of fats rich in linoleic acid, particularly corn, cottonseed and safflower oil, will produce a marked lowering of plasma cholesterol and other lipids.[5] Published studies, however, for the most part report the use of diets in which the *only* fat present was predominantly unsaturated.

During the past two years several studies have been carried out on the metabolic ward, and many observations have been made in ambulatory patients, using various ratios of saturated to unsaturated fat. One controlled study is shown in figure 1.

In this patient no significant decrease in plasma lipids was noted until 50 per cent of the predominantly unsaturated fat was substituted by safflower oil. This patient had no clinical evidence of vascular disease, despite his age (sixty-three) and mild diabetes. Diabetic control was perfect through the study. In this and other studies, weight remained steady, ± two pounds, unless otherwise noted.

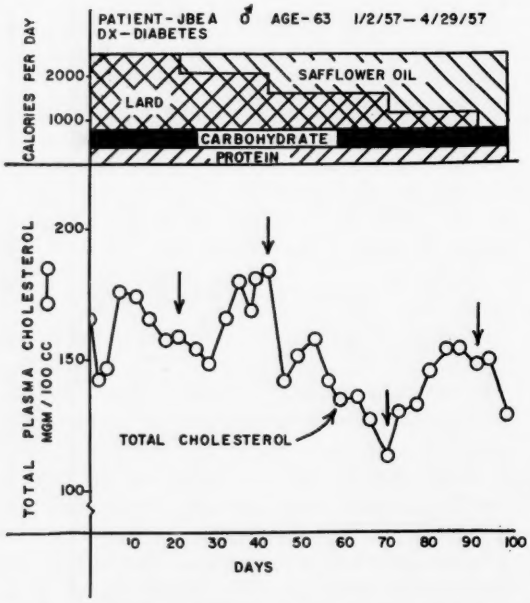


FIG. 1. Effects of different ratios of dietary unsaturated and saturated fat, respectively, upon levels of plasma cholesterol in a sixty-three-year-old male mild diabetic without evidence of vascular disease.

In table 1 are shown data on a group of patients selected only with regard to variety of diagnoses, who have recently been re-evaluated, and who have been followed for periods from several months to more than two years. With the exception of patient R.R. (a relatively young patient with coronary heart disease who probably has "familial hypercholesterolemia"), all patients have been studied during the intake of a Group I, II, or III diet.*

A group of fourteen apparently normal young male adults was studied for a period of approximately four months some two years ago. Initial plasma cholesterol values in these young men averaged more than 215 mg. per 100 ml. Revision of their diets to provide approximately 15 per cent of dietary fat as linoleic acid, with a total fat intake of 40 per cent to 50 per cent of dietary calories, was associated with maintenance of plasma cholesterol levels averaging less than 190 mg. per 100 ml.

From these observations in both normal and abnormal subjects, it would appear that one can make no sweeping generalizations as to ratios of linoleic acid to saturated fat which will result in a predictable level of plasma lipids, inasmuch as such ratios are dependent upon the intrinsic state of the individual who is consuming the diet.[6]

Linoleic acid as the major "hypocholesterolemic agent" in vegetable fats. The question has been raised as to the mechanism of lowering of the plasma lipids by a variety of vegetable fats. Among the entities present in or absent from vegetable fat which have been considered are: (a) the absence of cholesterol; (b) the presence

*Each of these diets contains approximately 58 gm. of linoleic acid. Group I contains a total of 100 gm. of fat (42 gm. from saturated and mono-unsaturated sources. Fish, fowl, and liver are permitted in the Group II diet).

Group III contains a total of 115 gm. of fat (52 gm. from saturated and mono-unsaturated sources. In addition to fish and fowl, some meat, dairy fat, and eggs are permitted).

All of the above figures are based upon a 2,000 calorie intake. Fat is increased or decreased proportionately in relation to calories.

In patients with insulin-dependent diabetes, the diets are modified so that 7 per cent vegetables replace some of the more concentrated carbohydrates.

Copies of the Group I, II and III diets may be found in the Appendix. These diets are discussed in more detail elsewhere.[3]

TABLE 1

Name	Age	Diagnosis	Diet group	Total plasma lipid Original Date	Level	On diet Date	Level	Total plasma cholesterol Original	On diet
E.S.	52	Xanthomatosis	I	5/26/58	2,844	9/23/58	704	680	213
N.C.	62	Gout, angina pectoris	II	9/20/56	—	10/7/58	838	270	198
W.W.	51	No demonstrable pathology	III	9/18/58	813	11/18/58	597	260	199
A.W.	49	Angina pectoris	I	12/3/57	1,016	3/6/58	625	305	148
			II	10/28/58	547				179
L.S.	62	Coronary insufficiency	II	3/4/58	1,041	11/18/58	978	268	218
D.L.	58	Coronary heart disease	II	8/25/58	1,022	10/2/58	866	280	199
R.R.	48	Coronary heart disease	80 gm. Ethyl Linoleate	6/10/58	1,125	10/16/58	1,013	362	300
R.S.	73	Peripheral vascular disease	II	5/7/57	950	10/7/58	876	239	174
T.L.	50	Peripheral vascular disease	I	7/22/58	869	10/28/58	681	274	198
D.B.	60	"	I	5/13/58	883	2/19/58	698	295	204
L.J.	54	Hypoparathyroid, arcus senilis	II	11/30/57	741	9/11/58	616	220	179

The policy followed with most patients is to place them initially on a Group I diet if the plasma cholesterol level exceeds 240 mg. per cent, or the total lipid exceeds 900 mg. per cent. Otherwise, the patient starts with the Group II diet, and "graduates" to a more liberal diet when his plasma cholesterol level has been below 200 mg. per cent (preferably below 180 mg. per cent) for two or more months. Some patients find it necessary to return to the more restricted diet because of a prompt and maintained rise in plasma cholesterol levels while on the Group II or Group III diets. Some individuals in whom the elevated lipid is an isolated abnormal finding (e.g., W.W., above) are placed on a Group III diet initially, regardless of the initial lipid values. If the patient fails to respond, a more rigorous program is adopted.

In more than 50 per cent of patients so followed, the decrease in plasma lipid levels is associated with apparent clinical improvement. This is particularly true of the patient with peripheral vascular disease.

Some patients refuse to adhere to a Group I diet (e.g., L.S., and D.L., above). In these, Group II usually represents a workable compromise, although normal lipid values may not be achieved.

Temporary use of formula diets containing very large amounts of linoleic acid is required in a few patients who fail to respond to the Group I diet.

of certain vegetable sterols; (c) the presence of certain vegetable phospholipids; (d) the nature of one or more of the fatty acids present; (e) the presence of trace materials.

The absence of cholesterol has been excluded as a major factor.[5a] Phospholipids, if they contain a sufficient quantity of unsaturated fatty acids, may produce a striking reduction. In our experience thus far saturated phospholipids fail to produce such an effect.[7]

Beveridge and his associates believe that vegetable sterols, particularly beta-sitosterol, are responsible to a significant degree for the cholesterol-lowering effect.[8] In our experience the vegetable sterols have a relatively weak and unpredictable effect of this sort.

Since the fatty acids of animal fats are predominantly saturated, and the fatty acids of most vegetable fats are predominantly polyunsaturated, with linoleic acid as the major component of the vegetable fats which lower cholesterol and other lipids, the question arises whether linoleic acid per se is capable of lowering plasma lipids. As reported previously[7] this is indeed the case. In figure 2 is a portion of a recent study in a young male with

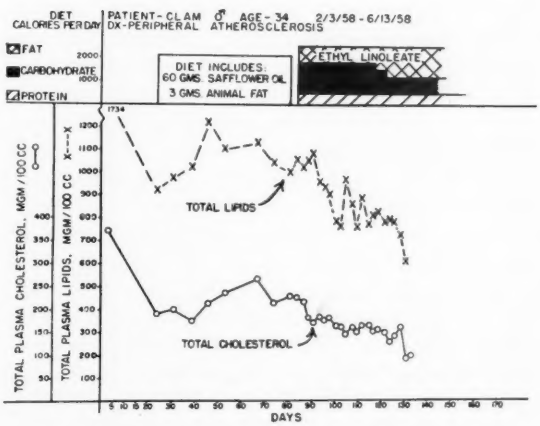


FIG. 2. Ethyl linoleate as the sole source of dietary fat was associated with a progressive decrease in plasma lipids in this young male with advanced peripheral atherosclerosis.

peripheral atherosclerosis in association with elevation of plasma cholesterol and of total lipids. Ethyl linoleate produced a greater fall in the plasma lipid levels than

had moderate amounts of natural sources of unsaturated fat. Linoleic acid, therefore, appears to be the most important single lipid-lowering component of vegetable fat. As to the possible hypocholesterolemic role of materials present in trace amounts in the vegetable fat, this cannot be excluded, but the observations regarding linoleic acid make such a possibility unlikely.

Effects of a "nonessential" unsaturated acid (oleic acid). The actual mechanism of the effect of dietary linoleic acid upon the plasma lipids is unclear at the present time. The three major possibilities appear to be (a) decreased cholesterol and phospholipid production; (b) increased excretion of the materials themselves or of their metabolites; and (c) increased destruction. Except to say that no adequate explanation is yet available, this subject will not be pursued further in this paper. A related unknown is the question whether the effect is a function of *essential* fatty acids,* per se, or whether it is a function in some way of the pharmocologic effect of a variety of fatty acids containing a sufficient number of unsaturated bonds, e.g. oleic, and polyunsaturated *nonessential* fatty acids.

Methodology has only recently been developed to the point where one is able to carry out determinations of fatty acid composition in fractions of the plasma lipids such as the cholesterol esters, the glycerides, and the individual phospholipids. Only cholesterol ester fatty acid composition will be considered in this report.

In figure 3 are observations in a male diabetic, aged thirty-four, during the course of a metabolic study in which oleate and linoleate were administered for stated periods of time in the amounts indicated.

Significantly higher levels of cholesterol were observed during oleate administration than during administration of equal amounts of linoleate. The relatively low cholesterol values during the second oleate period may have been related to linoleate stored in fat depots. The fatty acid composition of the cholesterol esters reflected the fat which was fed, i.e., the mono-enoic† acid content averaged more than 40 per cent during oleate feeding and less than 20 per cent during linoleate ingestion. Essentially, a mirror image of this resulted during linoleate feeding, at which time di-enoic acid predominated.

Additional work remains to be done with a variety

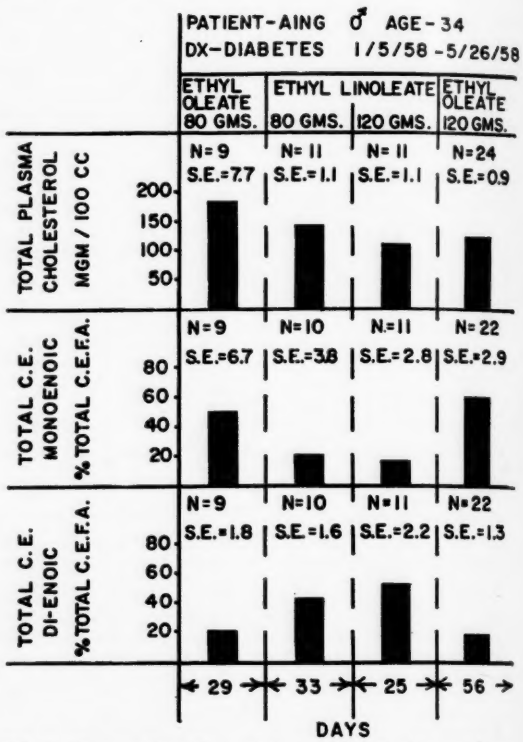


FIG. 3. Comparative effects of oleate and linoleate upon plasma lipids in a young diabetic. In this patient, as in all others included in this study, diabetic control and weight were constant throughout. C.E.=cholesterol ester. C.E.F.A.=cholesterol ester fatty acid. N=number of observations. S.E.=standard error.

of essential and nonessential unsaturated fatty acids before one can state categorically that satisfactory cholesterol-lowering is a function of *essential* fatty acids per se.

In figure 4 is shown a similar study in a sixty-one-year-old male with no clinical evidence of vascular disease. Oleate, again, was associated with high mono- and low dienoic acid in the cholesterol esters, and linoleate ingestion with the opposite pattern. Total lipids and cholesterol follow the same pattern in their response to unsaturated fat. No exceptions to this pattern have been noted thus far in nonatherosclerotic subjects.

In figure 5 are shown the mono- and di-enoic acid cholesterol ester values in a middle-aged female, again with no clinical evidence of vascular disease, who was maintained on a completely fat-free (hence high carbohydrate) formula diet. The fatty acid composition of the cholesterol esters was comparable with that seen in patients receiving oleate. The plasma cho-

*An essential nutrient is one which cannot be synthesized in significant amounts in the body, and whose absence from the diet will result in clinical and biochemical abnormalities.

†Mono-enoic (mono-unsaturated) acid is presumably synonymous under these conditions with oleic acid and di-enoic (di-unsaturated) acid with linoleic acid.

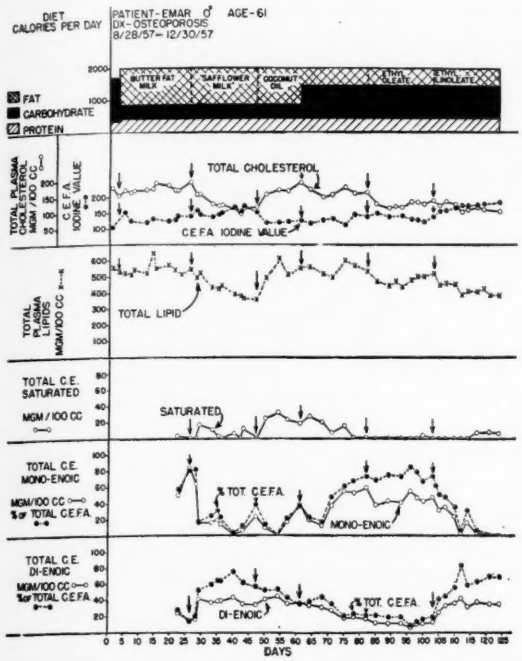


FIG. 4. Effects of different fats upon blood lipids. Linoleate feeding in natural or highly purified form is associated with increased cholesterol ester di-enoic acid and decreased mono-enoic acid. Oleate feeding produces the opposite pattern.

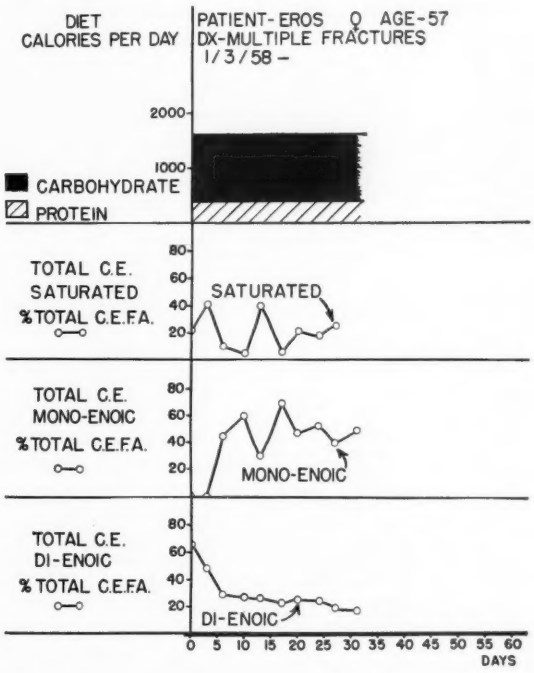


FIG. 5. Effects of high carbohydrate intake upon plasma cholesterol ester fatty acids in a middle-aged female with no evidence of vascular disease.

lesterol levels noted during oleate and carbohydrate feeding respectively also are very similar, but with diets containing large amounts of carbohydrate one usually notes a marked rise in the level of neutral fat. This rise may be transient, or may continue for an extended period of time. We have not observed such a rise in plasma neutral fat in any patient receiving appreciable amounts of ethyl oleate. Presumably it is related in some way to the high rate of lipogenesis in patients receiving the high carbohydrate, fat-free diets.

Comparative fatty acid composition of the plasma cholesterol esters as determined by gas chromatography in a normal young male and in a patient with extensive peripheral atherosclerosis is shown in figure 6. Significant differences are present. Even intensive linoleate feeding has so far failed to bring this patient's cholesterol linoleate to "normal."

Fatty acid composition of mono-, di-, and triglycerides and of individual phospholipids will be the subject of a later report.

Clinical changes in diabetics and nondiabetics with vascular disease. So long as one restricts his investigative efforts to the evaluation of changes in the pattern of blood lipids observed under controlled conditions, one is on firm ground. The evaluation of changes in clinical status introduces a host of problems. Our experience to date in this field deals with diabetics and nondiabetics who have atherosclerosis involving the cerebral vessels, the coronary vessels, and the vessels of the lower extremities, and with diabetics with characteristic retinal and renal vascular disease.

In evaluating atherosclerosis in the human subject, one obviously is at an extreme disadvantage in that he is unable to examine directly and periodically the intima of any given artery, and must therefore do his best to evaluate subjective and objective changes in circulation. At the present time, we make no attempt to evaluate objectively changes in cerebral circulation or in coronary circulation, inasmuch as the spontaneous variation of these conditions on a variety of regimens is well known.

In the case of peripheral atherosclerosis, the investigator is working in somewhat better territory, inasmuch as, in addition to changes in functional capacity of the individual suffering from intermittent claudication, he

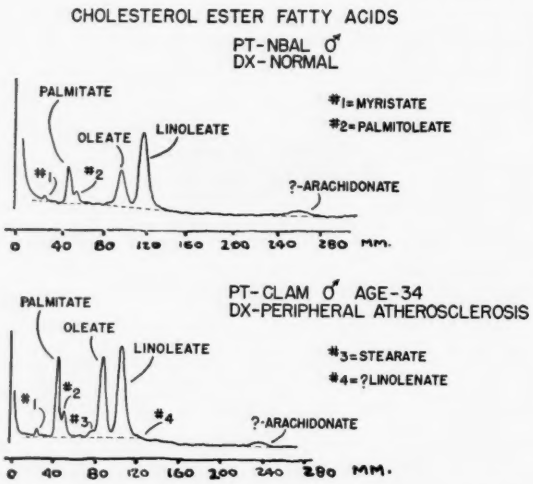


FIG. 6. Fatty acid composition of plasma cholesterol esters in a normal young male (NBal) and in a thirty-four-year-old male with extensive peripheral vascular disease (CLam). These patients were on similar diets when these observations were made.
The approximate per cent composition with reference to major fatty acid components is:

	NBal	CLam
Myristic	1.4	1.1
Palmitic	13.3	15.4
Palmitoleic	4.8	6.7
Stearic	1.4	2.0
Oleic	21.2	28.6
Linoleic	52.0	42.3
Arachidonic	6.0	3.8

can also record changes in pulsation of the arteries at any desired level in a given limb. Even in this area, however, one must interpret with great caution. At this time we can say only that in a group of patients with major impairment of the arterial circulation in the lower extremities, sufficient subjective and objective improvement has occurred to lead us to believe that there may be some relationship between the improvement and the feeding of nutritionally adequate diets which contain large amounts of fats rich in linoleic acid, and which are restricted in saturated fat. This will be the subject of a later report.

In the case of diabetic retinopathy and nephropathy one also is dealing with clinical conditions which are most difficult to evaluate critically except on the basis of long-term observations in sufficient numbers of people to have real statistical meaning. Because of the elevated blood lipids in a number of these patients, large amounts of polyunsaturated fatty acids have been used in the diet under both controlled and semicontrolled conditions. An early study of this sort (1953) was carried out in a patient who had retinal involvement of sufficient degree to make it impossible for him to read even large print. Over a period of less than two months, on a daily formula diet which contained 100 gm. of cottonseed oil, his retinopathy improved to such a degree that he was able to read ordinary print. Objective changes in the fundi corresponded to the functional improvement. This was the first instance in which a change of this sort was noted. No regression has occurred. At the time it was felt that the improvement was referable entirely to improved stabilization of his diabetes. However, within the past two years several other patients with diabetic retinopathy, one of whom failed to make a favorable response to hypophysectomy, appear to have had significant improvement of retinopathy during the intake of diets containing large quantities of unsaturated fat. Again, it must be emphasized that several years must pass before it will be possible to know whether these changes are coincidental, or related to the dietary changes.

DISCUSSION

Investigative procedures carried out in the human subject have the obvious advantage of elimination of species differences. In perhaps no other field is this more important than in diabetic and nondiabetic vascular disease. Production of characteristic diabetic retinal and renal disease in experimental animals approaches the impossible. Conditions similar to human atherosclerosis have been produced in a variety of experimental animals, but all of these animals follow very different patterns of response to nutritional and pharmacologic agents as compared to the human subject. For this reason, there is increasing acceptance of the concept that the problems of vascular disease will probably not be solved except by properly directed and controlled human studies.

The data presented in this paper appear to establish that linoleic acid administered either as purified ethyl ester or as naturally occurring fat, in sufficient quantity, in properly constructed diets, will reduce plasma lipids to normal levels. The amount of linoleic acid required appears to bear a direct relationship to the amount of saturated fat included in the diet. Linoleic acid requirement may also bear a significant relationship to the amount of atherosclerosis present.

The transition from evaluation of the effect of dietary entities upon plasma lipids, to the evaluation of the effect of such materials upon vascular disease is difficult. However, such evaluation is not impossible. The requisites are adequate measuring sticks and well-controlled studies of sufficient duration. The duration of observation of effects of unsaturated fat in diabetic

and nondiabetic patients with vascular disease is in no instance more than five years, and in the majority of instances, less than three. Our present impression is that improvement has occurred in some patients with atherosclerosis and with diabetic retinal and renal disease which was more than we would have anticipated in terms of the natural course of the disease. However, since it is well known that major fluctuations in these diseases can occur in individuals receiving no treatment, we believe it is appropriate at this time to say that no untoward effects appear to result when one prescribes diets containing large amounts of unsaturated fat for patients with such diseases, and it is not impossible that beneficial effects may be associated with such diets.

SUMMARY

Many vegetable fats lower plasma cholesterol and other plasma lipids. Linoleic acid appears to be the constituent of these fats which is responsible for such effects. Generally speaking, the greater the amount of saturated fat in the diet, the greater the amount of linoleic acid required to produce a desirable lowering of plasma lipids. Because of major individual differences in response in patients with vascular pathology, lipidoses, etc., it is impossible to set up a ratio of relationships between dietary saturated and unsaturated fatty acids which will apply generally.

In some diabetic and nondiabetic patients with vascular disease, subjective and objective improvement has been noted in association with the intake of diets containing large amounts of linoleate. It is still too early to state positively that such improvement is related directly to the diets employed.

The fatty acid composition of the diet specifically influences the composition of the plasma cholesterol esters. High linoleate intake results in markedly increased plasma cholesterol linoleate, and high oleate intake results in markedly increased plasma cholesterol oleate.

SUMMARIO IN INTERLINGUA

Acido Linoleic E Linoleato Dietari: Effectos In Subjectos Diabetic E Nondiabetic Con E Sin Morbo Vascular

Multe grassias vegetal reduce le contento de cholesterol e de altere lipidos in le plasma. Il pare que acido linoleic es le constituente de iste grassias que es responsabile pro le effecto mentionate. A generalmente parlar, quanto plus grande le quantitate de grassia saturate in le dieta, tanto plus grande le quantitate de acido linoleic requirite pro producer le desirate reduction in le contento de lipidos del plasma. A causa de major differentias individual in le responsa de patientes con pathologia vascular, lipoidosis, etc., il non es possibile fixar un proportion inter saturate e nonsaturate acidos grasse del dieta que pote esser usate in omne casos.

In certe patientes diabetic e nondiabetic con morbo vascular, un melioration subjective e objective esseva notate in association con le ingestion de dietas que contine grande quantitates de linoleato. Il esserea precoce asserer positivemente que ille melioration es relationate directemente con le dietas usate.

Le composition del acidos grasse in le dieta exerce specificamente un influentia super le composition del esteres cholesterolic del plasma. Un alte ingestion de linoleato resulta in marcate augmentos del linoleato cholesterolic del plasma, e un alte ingestion de oleato resulta in marcate augmentos del oleato cholesterolic del plasma.

ACKNOWLEDGMENT

Grateful acknowledgment is made to Carnation Company for supplies of Saf-Lac; Merck, Sharp and Dohme for digitonin used in methodology; The Borden Company for instant coffee used in metabolic studies; The Procter and Gamble Company and the Unilever Research Laboratories for generous supplies of ethyl linoleate.

This work was supported in part by grants from The Alameda County Heart Association, Carnation Company, The Nutrition Foundation, The Armour Company, and The National Institutes of Health.

REFERENCES

[1] Kinsell, L. W., Michaels, G. D., Partridge, J. W., Boling, L. A., Balch, H. E., and Cochrane, G. D.: Effect upon serum cholesterol and phospholipids of diets containing large amounts of vegetable fat. J. Clin. Nutrition *1*:224, 1953.

[2] Michaels, G. D., Wheeler, P., Fukayama, G., and Chin, H. P.: Column chromatographic fractionation of plasma lipids. Am. J. Clin. Nutrition *6*:604, 1958.

[3] Michaels, G. D., Wheeler, P., Fukayama, G., and Kinsell, L. W.: Plasma cholesterol fatty acids in human subjects as determined by alkaline isomerization and by gas chromatography. Annals of the New York Academy of Sciences *72*:633, 1959.

[4a] James, A. T.: The separation of long chain fatty acids by gas-liquid chromatography. Am. J. Clin. Nutrition *6*:595, 1958.

[4b] James, A. T., and Martin, A. J. P.: Gas-liquid chromatography. The separation and identification of the methyl esters of saturated and unsaturated acids from formic acid to n-octadecanoic acid. Biochem. J. *63*:144, 1956.

[4c] Lipsky, S. R., and Landown, R. D.: A new partition agent for use in the rapid separation of fatty acid esters by gas-liquid chromatography. Biochimica et Biophysica Acta (Amsterdam) *27*:666, 1958.

[5a] Kinsell, L. W., Partridge, J. W., Boling, L., Margen, S., and Michaels, G. D.: Dietary modification of serum cholesterol and phospholipid levels. J. Clin. Endocrinol. and Met. *12*:909, 1952.

[5b] Kinsell, L. W., Michaels, G. D., Cochrane, G. C., Partridge, J. W., Jahn, J. P., and Balch, H. E.: Effect of vegetable fat on hypercholesterolemia and hyperphospholipidemia. Observations on diabetic and nondiabetic subjects given diets high in vegetable fat and protein. Diabetes 3:113, 1954.

[5c] Ahrens, E. H., Jr., Bloomstrand, R., Hirsch, J., Insull, W., Peterson, M., and Tsaltas, T. T.: The influence of dietary fats on serum lipid levels in man. Lancet 1:943, 1957.

[6] Kinsell, L. W.: Essential fatty acids, lipid metabolism, and atherosclerosis. Lancet 1:857, 1958.

[7] Kinsell, L. W., Friskey, R., Splitter, S., Michaels, G. D.: Essential fatty acids, lipid metabolism, and atherosclerosis. Lancet 1:334, 1958.

[8] Beveridge, J. M., Connell, W. F., Firstbrook, J. B., Mayer, G. A., and Wolfe, M. J.: Effects of certain vegetable and animal fats on plasma lipids of humans. J. Nutrition 56:311, 1955.

[9] Olson, F., Splitter, S., Balch, H., Flynn, P. F., and Kinsell, L. W.: The use of diets containing large amounts of linoleic acid. Am. J. Clin. Nutrition 6:632, 1958.

APPENDIX

GROUP I, II AND III DIETS HIGH IN ESSENTIAL FATTY ACIDS

GROUP I

Approximately 2,000 calorie diet—high in linoleic acid, no meats allowed. P—80 gm., all from nonfat milk protein; F—100 gm.; C—200 gm.

Food	Household measure	Gram weight	Protein	Fat	CHO
Daily use following:					
Nonfat milk solids— or liquid skim milk	3/4 cup or 1 quart	90	32	0.9	47
Low calorie cottage cheese	2/3 cup	130	23.7	2.6	2.6
Oil { Safflower, Cotton, Corn, Soy }	1/4 cup or 4 tablespoons	60 cc.	—	55	—
Nuts { Walnuts—1/3 cup; Other nuts—1/3 cup }	2/3 cup	65	10	42	10
Cooked whole-grain cereal	1/2 cup	100	2	—	15
Fruit 20% or 10%	1 1/2 or 3 cups	300 or 600	—	—	60
Vegetable 20%	1 cup	200	4	—	40
Other than 20% vegetable	Use as desired cooked or raw	—	4	—	20
Low-fat bread	1 slice	25	2	—	15
		Total	77.7	100.5	209.6

Meal plan and instructions

Morning meal: 1 cup 10% fruit; ½ cup cooked whole-grain cereal; 1 tablespoon oil added to cereal just before serving; 1 cup skim milk; coffee or tea as desired with all meals.

Noon meal: Soup made with skim milk, vegetables and part of oil; 1/3 cup low calorie cottage cheese; cooked or raw vegetable, as desired, of nonstarchy group; 1/3 cup nuts, used with lunch or between meals with cheese or vegetables and fruit; 1 slice low-fat bread; 1½ tablespoons oil used in soup, vegetable or as salad dressing. Make salad dressing—do not use egg yolk—may use egg white—1 tablespoon dressing equals 2 teaspoons oil; ½ cup 20% fruit or 1 cup 10% fruit.

Evening meal: 1/3 cup low calorie cottage cheese; 1 cup 20% vegetable; other vegetables, as desired, cooked or raw; 1½ tablespoons oil used as salad dressing, or added to vegetables; ½ cup 20% fruit or 1 cup 10% fruit; 1/3 cup nuts used with meal or complete before bedtime; complete skim milk at evening meal or before bedtime.

Instructions: To simplify fruit and vegetable selection, consider banana, white and red grapes, and canned fruit with sugar as 20 per cent fruits; all other unsweetened fresh or canned fruit as 10 per cent.

Twenty per cent vegetables: corn, cooked beans, cooked rice, cooked macaroni, potato. All other vegetables in lower percentage groups (or less starchy varieties) are allowed as desired.

Low-fat breads: French (sweet or sour); Milwaukee

pumpernickel; English muffins; rye krisp.

Oils may be used in cooking; in salad dressing; added to cereals; combined with skim milk, if emulsifier is available. *Be sure* that *all* oil is used when combined with food and not left in bowl or cooking utensil.

Nuts are useful in adding variety to vegetables, fruits and cottage cheese. Also may be blended with skim milk, if blender is available. Walnuts (containing more linoleic acid) should supply one half of nuts, the remainder from mixed nuts, *not* commercially fried. If fried, fry in one of suggested oils at home.

This diet should be supplemented with multivitamins and iron. (It is especially low in iron.)

The approximate amount of linoleic acid in the diet is 58 gm., if one-half walnuts, one-half peanuts and safflower oil are used. If other nuts and oils are used, it will be about one-quarter to one-third less.

To make emulsion of oil and milk for one day, use: ¾ cup nonfat milk solids; 2 tablespoons oil; 1 teaspoon "special lecithin";* 3½ cups water. Place in bowl one cup water, lecithin, and oil. Use beater or blender; add milk solids and balance of water. Use as milk during day. Use the remaining two tablespoons of oil for cooking as previously instructed.

*Centrophil SC, Central Soya Company, Inc., Chicago.

GROUP II

Approximately 2,000 calorie diet—allowing fish and fowl in addition to fat-free milk proteins. High in linoleic acid. Approximately P—80 gm.; F—105 gm.; C—190 gm.

Food	Household measure	Gram weight	Protein	Fat	CHO
Daily use following:					
Nonfat milk solids— or liquid skim milk	1/2 cup or 2 1/2 cups	60	21	0.6	31
Low calorie cottage cheese	1/2 cup	100	20	2	2
Fish, fowl and liver (2x wk.)	4 oz. or 1/4 lb.	120	24	6	—
Oil { Safflower, cotton / Corn, soy	1/4 cup or 4 tablespoons	60 cc.	—	55	—
Nuts { Walnuts—1/3 cup / Other nuts—1/3 cup	2/3 cup	65	10	42	10
Cooked whole-grain cereal	1/2 cup	100	2	—	15
Fruit 20% or 10%	1 1/2 cups or 3 cups	300 or 600	—	—	60 —
Vegetable 20%	1/2 cup	100	2	—	20
Other than 20% vegetable	Use as desired cooked or raw	—	4	—	20
Low-fat bread	2 slices	50	4	—	30
		Total	87	105.6	188

Meal plan and instructions

Morning meal: 1 cup 10% fruit; ½ cup cooked whole-grain cereal; 1 tablespoon oil added to cereal just before serving; 1 slice low-fat bread (toasted); ½ cup skim milk; coffee or tea as desired with all meals.

Noon meal: ½ cup low calorie cottage cheese; cooked and raw vegetable as desired of nonstarchy group; 1/3 cup nuts—use with lunch or between meals with cheese, vegetable or fruit; ½ cup 20% fruit *or* 1 cup 10% fruit; 1 slice low-fat bread; 1½ tablespoons oil used in vegetable or salad dressing. *Make* salad dressing; use egg white; *omit* egg yolk. One tablespoon dressing equals 2 teaspoons oil; 1 cup skim milk—as drink or used as base for soup with vegetables.

Evening meal: Fish (any kind; those high in fat appear to have the "right kind" of fat insofar as the effect upon plasma lipids is concerned), fowl (remove fat and skin), or liver; ½ cup 20% vegetable; other vegetables as desired, cooked or raw; 1½ tablespoons oil used in cooking fish, liver or fowl and vegetables; ½ cup 20% fruit *or* 1 cup 10% fruit; 1/3 cup nuts—use with meal or eat before bedtime; complete skim milk at evening meal or before bedtime.

Instructions: Same as for Group I, except as follows: Allow lean fowl, both dark and light meat; remove skin and visible fat. To make emulsion of oil and milk for one day, use: ½ cup nonfat milk solids; 2 tablespoons oil; 1 teaspoon "special lecithin";* 2 cups water.

*Centrophil SC, Central Soya Company, Inc., Chicago.

GROUP III

Approximately 2,000 calorie diet—allowing lean meat, fowl, and fish in addition to fat-free milk protein. High in linoleic acid. Approximately P—75 gm.; F—115 gm.; C—170 gm.

Food	Household measure	Gram weight	Protein	Fat	CHO
Daily use following:					
Nonfat milk solids— or liquid skim milk	3 tablespoons or 1 cup	25 or 240 cc.	9 —	— —	13 —
Low calorie cottage cheese	1/2 cup	100	20	2	2
Lean meat, fish, or fowl	4 ounces	120	22	7	—
Oil { Safflower, cotton; Corn, soy	1/4 cup or 4 tablespoons	60 cc.	—	55	—
Nuts { Walnuts—1/3 cup; Other nuts—1/3 cup	2/3 cup	65	10	42	10
Butter or special margarine high in linoleic acid	3 teaspoons	15	—	12	—
Cooked whole-grain cereal	1/2 cup	100	2	—	15
Fruit 20% or 10%	1 1/2 cup or 3 cups	300 or 600	—	—	60
Vegetable 20%	1/2 cup	100	2	—	20
Other than 20% vegetable	Use as desired cooked or raw		4	—	20
Low-fat bread	2 slices	50	4	—	30
		Total	73	118	170

Meal plan and instructions

Morning meal: 1 cup 10% fruit; ½ cup cooked whole-grain cereal; 1 tablespoon oil added to cereal just before serving; 1 cup skim milk; 1 slice low-fat bread; 1 teaspoon butter; coffee or tea as desired with all meals.

Noon meal: ½ cup low calorie cottage cheese; cooked or raw vegetable, as desired, of nonstarchy group; 1½ tablespoons oil used in soup, vegetable or as salad dressing. *Make* salad dressing—do not use egg yolk—may use egg white. 1 tablespoon dressing equals 2 teaspoons oil; ½ cup 20% fruit or 1 cup 10% fruit; 1/3 cup nuts, used with lunch or between meals with cheese or vegetables and fruit; 1 slice low-fat bread; 1 teaspoon butter.

Evening meal: 4 oz. lean meat, fish, fowl or liver; ½ cup 20% vegetable; other vegetables, as desired, cooked or raw; 1 teaspoon butter; 1½ tablespoons oil used as salad dressing, or added to vegetables; ½ cup 20% fruit or 1 cup 10% fruit; 1/3 cup nuts with meal or complete before bedtime.

Instructions: Same as Groups I and II, except as follows: Allow lean fowl, both dark and light meat; remove skin and visible fat. Lean meat as lean beef round, lean veal, and liver; remove any visible fat.

Allow three eggs per week. When egg is used reduce cottage cheese to ¼ cup instead of ½ cup.

To make emulsion of oil and milk for one day, use: 3 tablespoons nonfat milk solids; 2 tablespoons oil; 1 teaspoon "special lecithin";* 1 cup water.

Place ½ cup water, lecithin and oil in bowl. Use beater or blender. Add milk solids and balance of water. Use as milk during day. Use the remaining two tablespoons of oil for cooking as previously instructed.

*Centrophil SC, Central Soya Company, Inc., Chicago.

Severe Diabetes with Remission

Report of a Case

Leonard J. Stutman, Capt., USAF (MC), and Jesse D. Hayes, Capt., USAF (MC), Dayton

The purpose of this report is to describe another instance of diabetes with remission which bears a remarkable similarity to a case[1] recently reported by Harwood. Both were young adults who were not known to have diabetes prior to the abrupt onset of symptomatic diabetic ketoacidosis and both remitted fully. In addition to this, we should like to record an interesting feature which may be helpful in diagnosis, utilizing intravenous tolbutamide as a diagnostic test for diabetes. Since Harwood's review of the literature, which did not show any other case of remission after severe acidosis in adults, Peck, Kirtley and Peck[2] have reported such an instance. Because of the great rarity of apparent cure or complete remission it was deemed worth-while to report the following case.

CASE REPORT

The patient, a nineteen-year-old white airman, had been apparently in good health until the middle of May 1957. At that time he was stationed at Lowry Air Force Base, Colorado. In April 1957, he observed that he had started to become noticeably thirsty and was losing weight. Early in May, he developed profound symptoms of diabetes: increasing polydipsia, polyuria and polyphagia. On May 27, 1957, he reported for examination and his urine contained 4-plus sugar, 4-plus acetone and 4-plus diacetic acid. CO_2—combining power of the blood was 14 volumes per cent; a blood glucose was 510 mg. per 100 ml.; peripheral blood count and chest film were normal.

There was no family history of diabetes. The patient had entered the service Dec. 26, 1956, and at that time had no evidence of any disease. His physical findings were within normal limits except for apathy and drowsiness. He responded to regular insulin, and after two days was placed on 30 units of NPH and a restricted diet 180 CHO, 95 fat and 70 protein. Later, because of increasing glycosuria, the dose of NPH insulin was increased to 65 units daily. The fasting and two-hour postprandial blood sugar returned to normal in several weeks and gradually the dose was reduced to 45 units of NPH daily when he seemed fairly stable and experienced no hypoglycemic reactions. After one month he was transferred to Wright-Patterson Air Force Base Hospital for Physical Evaluation Board action. At the time of admission to the second hospital he appeared in excellent control with a fasting blood sugar of 86 mg. per cent. Never under any conditions at our hospital did he have a fasting blood sugar over 95 mg. per cent while on insulin and diet 180 CHO, 95 fat and 70 protein. Because of low blood-sugar values and because he at times felt somewhat shaky, insulin was gradually withdrawn over a period of three weeks. Twenty-four-hour urine collections were negative for glucose at this time.

Several weeks after management on diet alone, a glucose tolerance test was done; this was within normal limits (figure 2, Number 1); two tolbutamide[4] tolerance tests revealed a relatively flat response (figure 1, Numbers 1 and 2). According to Unger[4] the blood glucose in a normal individual should drop 40 to 50 mg. per cent within the first twenty to forty minutes after intravenous tolbutamide.[4] This was confirmed in a control subject. Two glucose tolerance tests were done after the patient had been on a regular diet for several weeks (figure 2). It is interesting to note that in this period his glucose tolerance decreased. The patient at no time spilled any detectable sugar in his urine. These tests were carried out in a period of six weeks after admission to the Wright-Patterson Air Force Base Hospital, and at no time during these tests did the patient receive insulin. Tests for thyroid, adrenal and pituitary function were within normal limits (table 1).

After the patient was discharged from the hospital, it was

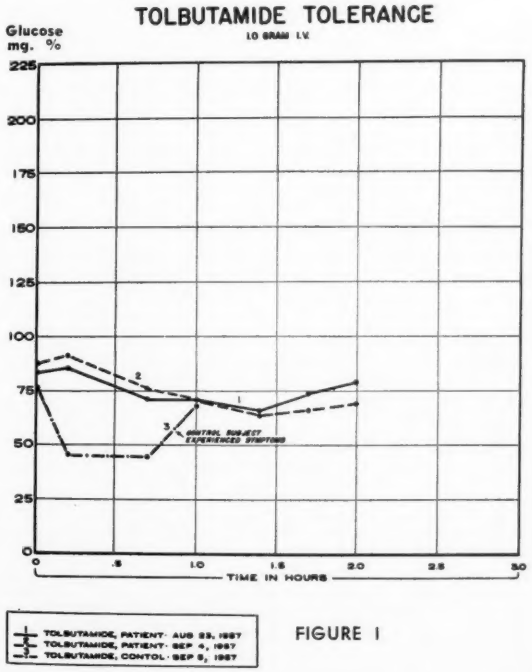


FIGURE 1

From the Department of Internal Medicine, United States Air Force Hospital, Wright-Patterson Air Force Base, Dayton, Ohio.

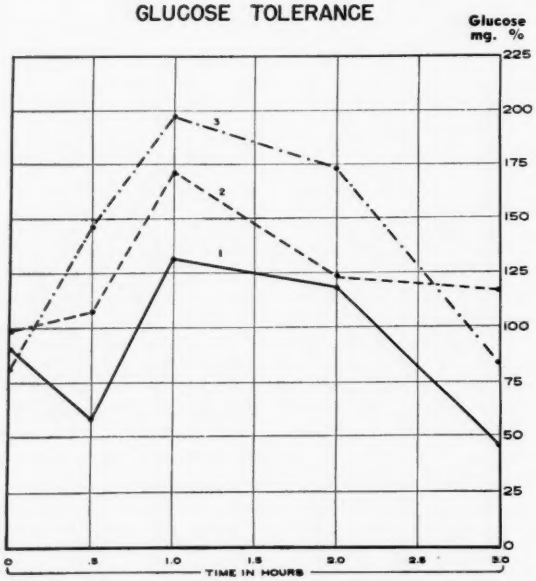


FIGURE 2

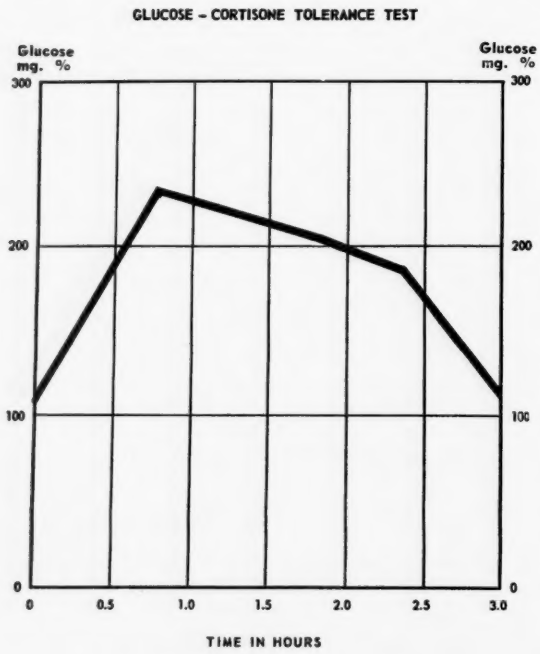


FIGURE 3

TABLE 1

Laboratory values

Protein bound iodine = 8.0 μg.
B.M.R. = (—6)
Cholesterol = 188 mg. per cent
A/G = 1.1 = 3.36/3.00, T.P. = 6.36

	Globulins per cent		Albumin per cent
Protein electrophoresis	Alpha one	3.8	52
	Alpha two	9.6	
	Beta	9.6	
	Gamma	25.0	

17-ketosteroids = 17.4 mg./24 hr.

suggested that a Fajans-Conn glucose-cortisone tolerance test be performed. The data appear in figure 3. It is obvious that according to the criteria of Fajans et al.[5] that this patient is a potential diabetic.

DISCUSSION

Joslin,[3] in his discussion of remissions, curability of diabetics and criteria for cures, feels that the tendency for diabetes is inborn and must remain so for life. In our case, there was no family history and no evidence for any precipitating factor for the onset of the diabetic ketoacidosis. Since we had a patient who was in remission as evidenced by the oral glucose tolerance, we thought it would be interesting to give him intravenous tolbutamide and see what the shape of his tolerance curve indicated. Unger by this means had been able to separate those diabetic subjects who have normal fasting blood sugars but have "diabetic" responses to the oral glucose test, from normal subjects because the latter have a sharp fall of 40 to 50 mg. per cent in blood glucose while the diabetics did not fall significantly. This decline was observed within ten to forty minutes. Our patient on two separate occasions had a flat curve. His glucose tolerance was normal when the first curve was done, but had decreased when the second curve was performed. This indicated that, even though the patient had an apparent remission, he is still a diabetic.

The use of an intravenous tolbutamide tolerance test has important implications in that it may help predict subjects who will develop diabetes in the future.

Several months after our patient had left the hospital, he developed lobar pneumonia and was ill seven days. During and after this infection there was no glycosuria and a fasting blood sugar obtained at the height of his disease was 95 mg. per 100 ml. Insofar as the stress of an infection usually aggravates existing diabetes we felt that this episode was significant in demonstrating the state of remission. Possibly if he continues for five years in such a manner the case could then be called a cure.

The Fajans-Conn provocative cortisone test and the tolbutamide tolerance test are both positive in this case and indicate that this patient still is a potential diabetic. Two fasting blood sugars determined under conditions without therapy or special diet one and one half years after the onset of diabetic ketoacidosis were 107 and 83 mg. per cent.

In the light of these tests most will agree with Joslin's view:

"The more I see of diabetes, the more strongly I think we should speak of remissions rather than of cures."

SUMMARY

1. A case of diabetic ketoacidosis with apparent remission has been described in a young male.

2. Severe infection did not bring on the symptoms of diabetes in the patient several months after he had an apparent remission from the diabetic acidosis.

3. Both the provocative cortisone and intravenous tolbutamide tolerance tests were positive for latent diabetes long after the patient was in a state of clinical remission.

SUMMARIO IN INTERLINGUA

Reporto De Un Caso De Remission in Diabete Sever

1. Es describite un caso de ceto-acidosis diabetic con remission apparente in un juvene masculo.

2. Un infection de grados sever non evocava le symptomas de diabete plure menses post le remission ab le acidosis diabetic.

3. Le test provocatori de cortisona e le test del tolerantia pro tolbutamido intravenose esseva positive pro diabete latente un longe tempore post que le patiente habeva attingite un stato de remission clinic.

ACKNOWLEDGMENT

The authors wish to thank The Upjohn Company of Kalamazoo, Michigan, for supplying the intravenous tolbutamide.

REFERENCES

[1] Harwood, Reed: Severe diabetes with remission: report of a case and review of the literature. New England J. Med. 257:257, Aug. 7, 1957, No. 6.

[2] Peck, Franklin B., Jr.; Kirtley, W. R.; and Peck, Franklin B., Sr.: Complete remission of severe diabetes. Diabetes 7:93-97, March-April 1958.

[3] Joslin, E. P., Root, H. F., White, P., and Marble, A.: The Treatment of Diabetes Mellitus. Philadelphia, Lea & Febiger, 1952, p. 272.

[4] Unger, Roger H., and Madison, Leonard L.: A new diagnostic test for early diabetes mellitus. Clin. Res. Proc. 5:187, April 1957.

[5] Fajans, S. S., and Conn, J. W.: An approach to the prediction of diabetes mellitus by modification of the glucose tolerance test with cortisone. Diabetes 3:296-304, July-August 1954.

The delineation of the complete genetic and biochemical pattern of man that I have envisaged involves, of course, many difficulties; but it also offers many challenges and promises important rewards. To give just one example, it would be of the utmost value in the elucidation of the basic mechanisms underlying heart disease to be able to formulate the metabolic interrelationships that must exist between the lipids and the purines. The relative levels of uric acid, cholesterol, and phospholipids have been shown to be related to the development of atherosclerosis and coronary artery disease.[1] Although the metabolism of each of these substances may be independently controlled by the activities of known genes, as in gout, xanthomatosis, and the lipidoses, respectively, some basic, underlying, genetic mechanism must surely exist which enzymatically conditions their interacting biochemical activities. A step in this direction may have been taken with the recent demonstration that familial amyloidosis represents an inherited aberrancy in lipopotein metabolism.[2]

Laurence H. Snyder, in "Fifty Years of Medical Genetics," from *Science*, Jan. 2, 1959.

[1] M. M. Gertler and P. D. White, *Coronary Heart Disease in Young Adults* (Harvard Univ. Press, Cambridge, Massachusetts, 1959).

[2] J. G. Rukavinia, W. D. Block, C. E. Jackson, H. F. Falls, J. H. Carey, A. C. Curtis, Medicine 35:239, 1956.

Neuropathic (Charcot) Joints Occurring in Diabetes Mellitus

W. M. Sheppe, M.D., Wheeling

In 1953 the author described the occurrence of advanced bony destruction of the metatarsals and phalanges in the foot of a patient exhibiting many other evidences of diabetic neuropathy.[1] This was considered to be a Charcot type of joint involvement observed in syphilis, syringomyelia, leprosy and diabetes mellitus. More recently we have observed two additional patients with Charcot joints which exhibited features of unusual interest.

In Case 1 the bony destruction apparently was initiated by damage done by bacterial invasion entering via an ulcerated fungus infection located between the fourth and fifth toes of the involved foot. The extensive cellulitis and edema of the foot and leg delayed the establishment of the true diagnosis for at least four months.

In Case 2 joint involvement occurred in a young diabetic as part of an insidiously developing diabetic neuropathy. This case is notable for the very extensive destruction of the bones of the left foot and the development of this severe complication at an unusually early age (twenty-six years).

Survey of the literature[1-7] indicates that approximately 100 such cases representing a manifestation of diabetic neuropathy have been reported, the majority appearing in the fifth and sixth decades.

CASE REPORTS

Case 1. Mrs. A. P., aged fifty-eight, was admitted to the hospital with marked swelling of the left foot and leg extending halfway to the knee. An ulcer, with foul-smelling exudate, was noted between the fourth and fifth toes. The skin of the leg and foot was tense and shiny, and the temperature was 103° F. The patient's diabetic control was poor and apparently had been for some time. Pressure on the foot and leg failed to produce any complaint of pain. The patellar and Achilles reflexes were absent and there was loss of touch, pain, vibration and position sensation in the feet. Examination of the spinal fluid disclosed a protein content of 95 mg. per 100 ml. as the only abnormality.

Presented by title at the Eighteenth Annual Meeting of the American Diabetes Association in San Francisco June 21-22, 1958.

From the Department of Internal Medicine, The Wheeling Clinic, Wheeling, West Virginia.

Examination of the ocular fundi revealed extensive diabetic retinopathy with marked impairment of vision.

X-ray examination of the left foot indicated "marked fragmentation of the fourth and fifth metatarsal bones."

The foot and leg were treated conservatively by continuous warm boric acid compresses, and the administration of oxytetracycline. Vitamins and iron were given orally for the existing anemia. Good diabetic control was established, and the patient was discharged but only to be readmitted three weeks later with recurrent swelling of the foot and leg and spontaneous rupture of the skin on the medial side of the foot. Foul-smelling purulent drainage was profuse. There was still very little complaint of discomfort.

Nocturnal diarrhea had been noted during the period between admissions.

The foot was widely incised by Dr. C. B. Buffington, and through-and-through soft rubber tissue drains were inserted. A large amount of purulent material was evacuated through the drains, the process being aided by irrigation. The tissues appeared to be well supplied with blood. Edema and redness of the foot and leg subsided, and good diabetic control was attained. The patient was discharged on a regimen including foot baths twice daily and the ingestion of oxytetracycline.

She was seen on an ambulant basis at monthly intervals. Drainage from the incisions gradually ceased, and healing of soft tissues occurred. In spite of the marked involvement of the soft parts and the extensive bone destruction, the patient continued to walk on the foot without complaint of pain.

Serial X-ray examinations indicated that the bony destruction without regeneration which was first noted on March 26, 1957, in the fourth and fifth metatarsals had on July 23, 1957, involved the tarsus, the ankle joint and the lower end of the tibia (figure 1).

Early in 1958 the results of the X-ray examination were reported as follows:

"Examination of the left foot shows extensive destruction of all bones of the tarsus and also of the fifth metatarsal. There is complete destruction of the joint between tibia, fibula and astragalus with destruction of the major portion of the last bone and the distal end of the tibia. Many bone fragments lie in the area of the tarsus.

Diagnosis: Charcot's disease involving fifth metatarsal, tarsus, and left ankle joint."

Because of the marked deformity of the foot and the accompanying bone destruction, below-the-knee amputation was proposed, but rejected by the patient, who continued to have good diabetic control.

Comment: To re-create the course of events in this case we can assume that this poorly controlled fifty-eight-year-old diabetic patient developed diabetic neuropathy as evidenced by

FIG. 1. Note destruction of the bones of the tarsus and the involvement of the ankle joint and lower end of the tibia (Case 1).

loss of patellar and Achilles reflexes, nocturnal diarrhea, and sensory abnormalities all accompanied by extensive diabetic retinopathy. A neglected fungus and bacterial infection spread rapidly through the soft tissues of the foot and leg. The lysis and fragmentation of bone was instigated or accelerated by the insult of gross bacterial invasion. This process, accompanied by joint destruction, proceeded steadily and inexorably over a period of eighteen months. Massive edema and extensive cellulitis obscured the clinical picture and resulted in a tardy recognition of the true type of bone involvement.

Case 2. This patient, now in his twenty-sixth year, was first seen at the age of thirteen years. His diabetes has always been severe and extremely unstable.

During the ensuing thirteen years there were numerous episodes of hypoglycemia, acidosis and coma entailing numerous admissions to the hospital. In spite of the best efforts of the parents, the patient became morose, resentful of medical and parental control and complicated his already precarious status by his poor cooperation. His work potential has been very low and his behavior has at times bordered on the psychotic. During 1955 and 1956 the patient spent two months in a psychiatric institution and three months on the medical service of a general hospital. A swelling of the left foot was noted at the latter institution, but it was attributed to "phlebitis." No X-ray examination of the foot was made. He was readmitted to the Ohio Valley General Hospital, Wheeling, West Virginia, on Aug. 2, 1956, during a period of severe acidosis and precoma.

On this admission the patient was observed to be very pale, and the abdomen was markedly distended with fluid. The liver and spleen were moderately enlarged, and a small infected ulceration was noted on the right great toe.

The left foot was markedly swollen, the swelling extending to the level of the malleoli. There was absolutely no painful sensation elicited on deep pressure, on weight bearing or on walking. The patient denied history of injury to the foot (figure 2). Touch, pain, vibration and position sensation were greatly diminished. Patellar and Achilles reflexes were absent.

Severe secondary anemia (red blood count 2.3-million/mm.; hemoglobin 7.5 gm.) was present, and the cephalin-flocculation was strongly positive. Serologic examination of blood and spinal fluid was negative for syphilis. Spinal fluid protein of 75 mg. per 100 ml. was reported. Bromsulphalein liver function test was reported as 18 per cent retention of dye in one-half hour and 8 per cent in one hour. Serum bilirubin was found to be 0.6 to 0.9 units. Total serum protein was recorded as 4.6 gm. per 100 ml., the A/G ratio being 1.6/1.4, and thymol turbidity was reported at 8 units. The cardiovascular findings were essentially normal. The urine contained albumen and casts, and the blood urea nitrogen was 38 mg./100 ml. There was no evidence of cardiovascular-renal failure.

Rather severe diarrhea (three to eight stools per day) was present but gradually subsided. The results of X-ray examination of the gastrointestinal tract, including opaque enema, were entirely normal.

X-ray examination of the left foot was reported as follows: "There is moderately extensive bony fragmentation and eburnation with loss of normal cortex in the region of the tarsal metatarsal area. There appears also to be some element of subluxation toward the lateral aspect of the metatarsals on the tarsals. Increased bone production is also noted. There is some flattening of the metatarsal phalangeal joint of the second digit. Changes are considered typical of so-called Charcot neuroarthropathy. With the history of diabetes, this rather rare type of neurotrophic joint would appear to be the best possibility. It should be pointed out that other types of neurotrophic joints should be ruled out.

FIG. 2. Painless enlargement of the left foot and ankle (Case 2).

"Impression: Extensive changes about the tarsal metatarsal joints and bones, left foot, consisting of bony fragmentation and eburnation with associated subluxation. Neuro-arthropathy of diabetes suspected" (figure 3).

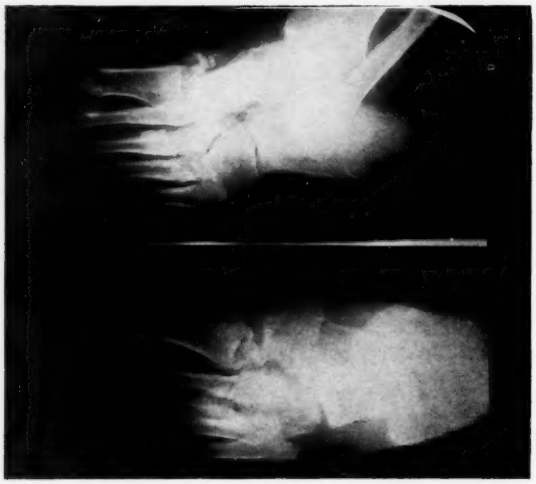


FIG. 3. Subluxation of the lateral aspect of the metatarsals on the tarsals with marked flattening of the arches of the foot (Case 2).

By abdominal paracentesis 4,000 cc. of ascitic fluid were removed. The diabetic control improved, and after two transfusions of whole blood the patient was discharged only to be readmitted four months later. At that time he again exhibited marked ascites, secondary anemia and a loss of diabetic control. The report of the X-ray examination of the left foot was as follows:

"Re-examination of the left foot shows changes similar to those described four months prior. In addition, there has been some further fragmentation of the bases of the fourth and fifth metatarsals. The lateral subluxation of the metatarsals on the tarsals may be slightly more exaggerated. Lateral examination at this time shows that the transverse arch of the foot is almost completely nonexistent and that a marked pes planus exists. The bony structure of the right foot is not remarkable. Calcification of the vessels of the foot is noted. This is from a minimal to moderate degree.

"Impression: Some slight increase in the amount of fragmentation and eburnation of the tarso-metatarsal area on the left foot. This is again thought to be consistent with a picture of diabetic neuro-arthropathy."

His final hospital admission was on Feb. 14, 1958. At that time ascites was tremendous (12,000 cc.) and hematemesis, presumably from esophageal varices, was severe and persistent. The red blood count was 2.2-million/mm. and the platelet count 60,000/mm. Blood and marrow studies by Dr. C. H. Hiles strongly suggested the presence of hypersplenism.

Following abdominal paracentesis, Dr. D. W. Dickinson, by means of a splenoportogram, demonstrated an intrahepatic portal obstruction. The splenic and portal veins were shown to be tremendously dilated with reversal of venous circulation into the gastrohepatic venous plexus. A splenectomy and splenorenal shunt was performed by Dr. C. D. Hershey. The liver was dark, greenish red in color, markedly enlarged (all lobes) but was not nodular. A biopsy was not performed. Six months after recovery from the surgical procedure the intervals between the performance of abdominal paracentesis have increased from about ten days to three months. Unexpectedly there has been a remarkable improvement in carbohydrate tolerance, the insulin requirement having fallen from 120 units to 16 units daily.

The left foot remains "club like" in appearance, *painless*, and bone destruction seems to have ceased.

DISCUSSION

It is apparent that the bony changes which develop into a characteristic neuropathic joint may develop insidiously, may occur following repeated minor traumas, or after severe soft tissue infection. Case 1 illustrates that gross infection may cause difficulty in the diagnosis of Charcot joints in diabetic patients. In such an instance the bone changes may be erroneously diagnosed as an ordinary osteitis or osteomyelitis. Evidences of diabetic neuropathy, the "club foot" appearance, the painlessness of the deformity and the extensive bone destruction best described as "lysis" should eventually establish the correct diagnosis. It is emphasized that every diabetic exhibiting a swollen foot should have the benefit of an X-ray examination.

The ages of all the reported cases are not given but it appears that the development of a Charcot joint as the result of diabetic neuropathy is rare prior to the fifth decade.

The second patient is of interest because he was only twenty-six years old when the diagnosis was established.

All three patients with this complication have had associated retinopathy, a very severe secondary anemia, elevated spinal fluid protein, and prolonged neglect of the details of diabetic management.

DIAGNOSIS

Recognition of these lesions is not difficult provided they are kept in mind. There are always evidences of diabetic neuropathy to be found. In our experience the circulatory status of the affected extremity has remained unimpaired and surgical incision, when performed, produces the usual amount of bleeding. The presence of a swollen or peculiarly deformed joint, usually unilateral, which is surprisingly painless, should strongly suggest the existence of a neuropathic joint. The great majority of the reported cases involved the feet. The "club foot" appearance, when taken in conjunction with the radiologic findings, merits the appellation "a bag of bones."

On X-ray examination one is impressed by (a) the appearance of lysis of the bones with marked fragmentation; (b) the absence of bone regeneration; (c) the marked tendency to disarticulation and destruction of the joints; and (d) the complete flattening of the longitudinal arch of the foot.

TREATMENT

The medical treatment of individuals exhibiting neuropathic joints is naturally limited to accurate control of the diabetes. As the process is practically painless, analgesics are usually unnecessary. Due to their severe secondary anemia, whole blood transfusions were administered to our three patients.

Lumbar sympathectomy has been performed in some reported cases. The procedure is highly recommended by Parsons and Norton[4] on the basis of good results secured in four cases. The authors suggest that only in the absence of infection can maximum results be expected from sympathectomy. Biedelman and Duncan[5] also feel that sympathectomy has been of value. Evaluation of the result obtained from sympathectomy in one of our series of three cases has been inconclusive.

In the presence of gross infection, as in Case 1, radical through-and-through incision is in order with establishment of tube drainage and frequent irrigation.

The performance of an arthrodesis on an uninfected neuropathic joint has been discussed, but the results obtained are not impressive.[6] The use of a short leg brace and special shoes may be of value in selected cases. The immense enlargement of the joint constitutes a cosmetic defect which would seem to be intolerable. However, because of the fair stability of the quiescent joint, despite its loss of normal structure, and because of the relative painlessness of the joint, none of our patients has desired amputation.

SUMMARY

Two cases are reported illustrating the development of lysis and absorption of metatarsals, phalanges, tarsal bones and distal end of the tibia in severe complicated diabetes mellitus. These changes are secondary to diabetic neuropathy and are comparable to those occurring in other diseases producing neuropathic joint lesions. One case was complicated by gross infection and the other was characterized by a very early age of onset.

Both cases had uniform evidence indicating diabetic neuropathy, notable freedom from pain in spite of gross deformity, and no impairment of the circulation in the involved extremities.

SUMMARIO IN INTERLINGUA

Articulationes Neuropathic (Charcot), Occurrente In Diabete Mellite

Es reportate duo casos que illustra le disveloppamento de lyse e absorption de metatarsales, phalanges, tarsales, e termino distal del tibia in complicate diabete mellite de grado sever. Iste alterationes es secundari a neuropathia diabetic e pote esser comparate con le alterationes occurrente in altere morbos del typo producente neuropathic lesiones articular. Un del duo casos esseva complicate per infection grossier. Le altere esseva characterisate per un precocissime etate de declaration.

In ambe casos, indicationes de neuropathia diabetic esseva uniformemente evidente. In ambes, il habeva un notabile absentia de dolor in despecto de grossier deformitates e nulle obstruction del circulation in le extremitates afficite.

ACKNOWLEDGMENT

The author is indebted to Dr. W. K. Kalbfleisch, Department of Roentgenology, The Wheeling Clinic, and to Dr. A. K. Butler, Dr. J. L. Curry, and Dr. A. J. Barger, Department of Roentgenology, Ohio Valley General Hospital, Wheeling, West Virginia, for interpretation of the roentgenograms.

The medical and surgical consultants mentioned in the text are members of the staff of The Wheeling Clinic.

ADDENDUM

Since this report was prepared, two other cases of Charcot joints have been observed at The Wheeling Clinic, bringing the series to a total of five. The roentgenologic appearance of the feet in the last two cases was essentially as described above.

REFERENCES

[1] Sheppe, W. M.: Neuropathic (Charcot) joints occurring in diabetes mellitus. Ann. Int. Med. *39*:625, 1953.

[2] Bailey, C. C., and Root, H. F.: Neuropathic foot lesions in diabetes mellitus. New England J. Med. *236*:397, 1947.

[3] Joslin, E. P., Root, H. F., White, P., and Marble, A.: Treatment of Diabetes Mellitus. Philadelphia, Lea and Febiger, 9th ed., 1952, p. 484.

[4] Parsons, H., and Norton, W. S.: The management of diabetic neuropathic joints. New England J. Med. *224*:935, 1951.

[5] Beidelman, B., and Duncan, G. G.: Charcot joints and infectious vascular lesions of bones in diabetes mellitus. Am. J. Med. *12*:43-52, 1952.

[6] Johnson, E. W.: The surgical management of diabetic complications. West Virginia Med. J. *54*:157, May 1958.

[7] Parsons, H., and Norton, W. S.: Personal communication.

The Influence of Diet upon Insulin Dosage in Diabetes Mellitus

Marios C. Balodimos, M.D., Boston

The average daily insulin secretion of the pancreas of a normal person has not been established. From experiments in animals and deductions based upon their body weight, the insulin need of pancreatectomized humans has been theoretically estimated to be about 200 units.[1] In contrast, as a result of total pancreatectomies in nondiabetic human subjects the insulin need was found to be usually not more than 40 to 50 units daily. Indeed these patients are rather sensitive to insulin.[2-4] In a diabetic the requirement for exogenous insulin is variable, ranging from none to 30, 60, or in unusual cases 100 or more units daily. Attempts have been made to classify diabetics according to the amount of insulin required to control the disease, but so many extraneous factors must be considered that classification on this basis is unsatisfactory.

The importance of a carefully measured diet in diabetes is generally accepted, but it is not always appreciated by the patients and even by some physicians, because of the lifesaving, life-prolonging action and easy administration of insulin. The experience of older physicians, who treated diabetics for years before the discovery of insulin, is probably the best evidence for the importance of dietetic control in diabetic therapy. The present data are offered to emphasize this principle.

In recent years patients have come to the clinic with histories of taking many units of insulin for control. Yet after a short period in the hospital much smaller dosages proved adequate. For this reason we studied the case histories and hospital course of 100 consecutive diabetic patients, who reported that prior to admission they took 80 or more units of insulin daily for periods of weeks to months. Patients admitted for treatment of diabetic acidosis and coma and pregnant diabetic women temporarily requiring large doses were excluded. The underlying cause for this relatively high insulin requirement was established and the cases were accordingly divided into three major groups. An analysis of the 100 patients as to age, sex, onset and duration of diabetes is presented in table 1.

In group I (thirty-six patients) were included all cases with a recognized cause for such a high dose. Insulin resistance, requiring several hundred units daily, occurred in nine patients. The possible etiology and the management of this condition have been reviewed by many others.[5-11] Twelve patients had an infection or gangrene as explanation of the unusual insulin requirement. The hospital course of these patients depended on the course of their infection and insulin decreased only when this came under control. In fourteen patients the dose during controlled hospital conditions remained the same. It was felt that this was due to the nature and severity of their disease. Hemochromatosis, lymphoma or a hidden infection may at times be revealed by the necessity for an increase of the insulin dose. Hyperthyroidism was the cause in the last patient of group I and the insulin dose was lowered when the primary condition was controlled. Hypersecretion of other antagonistic hormones, treatment with cortisone, or liver disease may at times be causes for high insulin doses, although we did not have patients in these categories.

The largest group (group II—forty-five patients) while in the hospital under controlled conditions and

TABLE 1

Diabetic patients taking 80 units or more of insulin daily

Age group	Duration of diabetes (in years)		Group I (resistance, infection, etc.)	Group II (excess of food)	Group III (not hospitalized)
Juvenile diabetics*	up to 5 years		3	5	2
	5-15		2	4	2
	above 15		6	4	1
Nonjuvenile diabetics	up to 5 years		2	4	2
	5-15		1	10	1
Age 15-40	above 15		0	2	0
Nonjuvenile diabetics	up to 5 years		2	1	1
	5-15		9	9	6
Age above 40	above 15		11	6	4
Total		Men	16	25	10
		Women	20	20	9
			36	45	19

*Juvenile diabetic: by definition,[12] every person with onset of diabetes before age fifteen, irrespective of present age.

From the Joslin Clinic and the New England Deaconess Hospital, Boston.

diet developed low blood sugars and hypoglycemic reactions and the insulin dose required drastic reduction. In these patients no obvious reason for the large dose could be found except excess food intake. An increase in insulin had been substituted for a controlled diet. They were eating a more or less free diet and tried to "cover" the glycosuria by additional insulin. At other times they were in insulin reaction and were forced to overeat to cover the excess of insulin they had taken earlier in the day. They lived in a vicious cycle and increased the insulin dose for the excess food and the food for the excess insulin. On admission these patients were taking an average of 99 units of insulin daily, with range from 80 to 160 units. After a few days' hospitalization they were discharged on an adequate diabetic diet, according to standard rules[12] and an average insulin dose of 56 units (range from 6 to 100). In other words, 43 per cent of the insulin they had been taking was not necessary, if they followed the prescribed diet. When subsequently followed up some of these patients had increased their food intake again and were forced to increase their insulin.

Some physicians argue that there can be no harm in taking enough insulin to cover a free diet. It is true that, unlike the oral hypoglycemic agents, insulin can cover practically any amount of food, if given in large enough doses. But excess of food leads to overweight and obesity, bad companions to diabetes. Actually twenty-nine patients out of the forty-five in this group were found to be up to 90 lb. (41 kg.) above their estimated optimal weight. Others probably believe that by taking "plenty" of insulin they would not need to bother about their diet and could also avoid the complications of the disease. Unfortunately this is not so. At least fifteen out of the forty-five patients had diabetic retinopathy and five had clinical evidence of diabetic nephropathy (Kimmelstiel-Wilson disease).

Aside from the possibility of weight gain and the liability to insulin reactions, with their added dangers,[13,14] there are also other disadvantages to excessive dosage, viz.: the cost of needless insulin; the need for special syringes for doses over 80 units; discomfort from the large volume of fluid injected; varying absorption. Furthermore lipodystrophies may be favored. Insulin resistance with its problems in management, including the high mortality (41 per cent reported[11]) may be encouraged by these needlessly high doses.[15] Another consideration is that many of these cases might respond to treatment with the sulfonylureas and they are not considered only because of this factitiously high dose. Actually a few were controlled by tolbutamide when placed under controlled conditions and a restricted diet.

The analysis of the patients in table 1 shows a fairly good distribution in all groups and no preponderance of any particular type. They included patients of the obese-elderly, insulin-insensitive type as well as of the juvenile, insulin-sensitive type, all with one common factor—excess food intake.

The patients in group III (nineteen cases) were seen as outpatients. In most of these the large dose was obviously due to excess food (at least eleven patients). They were given a diabetic diet and advised to decrease their insulin dosage gradually.

The dose of "80 units and above" in this study was taken arbitrarily; similar results would be obtained by selection of a lower level. Eighty units was found more frequently than other doses, because it is easy for such patients to take a "syringeful" of insulin in the morning, hoping to forget for the rest of the day that they have diabetes!

SUMMARY

An evaluation of the causes of high insulin dosage in a group of 100 diabetic patients, who came to the Joslin Clinic taking 80 units or more of insulin daily, showed that excessive food intake was the most frequent. The possible disadvantages and dangers of this type of diabetes control are discussed.

SUMMARIO IN INTERLINGUA

Le Influentia Del Dieta Super Le Dosage De Insulina In Diabete Mellite

Un evalutation del causas de alte dosages de insulina requirite per un gruppo de 100 patientes diabetic visitante le Clinica Joslin—illes prendeva 80 unitates de insulina o plus per die—monstrava que excessos in le ingestion de alimentos esseva le plus frequente. Le possibile disavantages e periculos de iste typo de controlo de diabete es discutite.

REFERENCES

[1] Root, H. F.: Insulin resistance and bronze diabetes. New England J. Med. *201*:201-06, 1929.

[2] Waugh, J. M., Dixon, C. F., Clagett, O. T., Bollman, J. L., Sprague, R. G., and Comfort, M. W.: Total pancreatectomy: A symposium presenting four successful cases and a report on metabolic observations. Proc. Staff Meet. Mayo Clin. *21*:25-46.

[3] Warren, K. W.: Some aspects of total pancreatectomy. Lahey Clin. Bull. 7:86-96, 1951.

[4] Dituri, B.: Insulin-resistant diabetes after total pancreatectomy. Report of a case. New England J. Med. *251*:13-16, 1954.

[5] Martin, W. P., Martin, H. E., Lyster, R. W., and Strouse, S.: Insulin resistance. J. Clin. Endocrinol. *1*:387-98, 1941.

[6] Smelo, L. S.: Insulin resistance. Proc. Am. Diabetes Association *8*:77-111, 1948.

[7] Davidson, J. K., and Eddleman, E. E.: Insulin resistance. Arch. Int. Med. *86*:727-42, 1950.

[8] Berne, R. M., and Wallerstein, R. S.: The role of anti-

bodies in insulin resistance. Report of a case. J. Mt. Sinai Hosp. *17*:102-111, 1950.

[9] Dolger, H.: The management of insulin allergy and insulin resistance in diabetes mellitus. M. Clin. N. America *35*:783-90, 1952.

[10] Kaye, M., McGarry, E., Rosenfeld, I.: Acquired insulin resistance (a case report). Diabetes *4*:133-37, 1955.

[11] Hampton, A. G., Jr., Hunt, W. B., Jr., and Mulholland, H. B.: Insulin resistance. J.A.M.A. *161*:788-93, 1956.

[12] Joslin, E. P., Root, H. F., White, P., Marble, A.: The Treatment of Diabetes Mellitus. Philadelphia, Lea & Febiger, 9th ed., 1952.

[13] Zucker, H. D.: Cardiac and vascular accidents in insulin treated diabetics. Trans. Amer. Coll. Cardiol. *2*:96-103, 1952.

[14] Jensen, B.: Ueber akute und chronische Insulinschaeden bei der Zuckerkrankheit. Wien. klin. Wchnschr. *68*:141-43, 1956.

[15] Brentano, C.: Insulinresistenz als Folge von Insulinueberdosierung und ihre Behandlung mit Thiourazilverbindungen. Zschr. klin. Med. *152*:371-90, 1954.

Trans-Acids as Essential Fatty Acid Antagonists

The possibility of finding competitive inhibitors to the essential fatty acids (EFA) has occurred to many workers interested in fat metabolism. Thus, such inhibitors might be used to prevent certain types of rapid or abnormal growth as in tumors (I. Smedley-MacLean and L. C. A. Nunn, *Biochem. J.* 35:983, 1941), or might be formed during hydrogenation of vegetable oils to the detriment of the users of these products (H. M. Sinclair, *Lancet* 270:381, 1956). However, the search for this type of inhibition has not been rewarding. No enzyme in which the essential fatty acids have been shown to play a definite role has been obtained from animal sources; attempts to produce inhibition in the usual test animals are complicated by their relatively large stores of essential fatty acids and their ability to alter or destroy the postulated inhibitors.

The logical compounds for trial as inhibitors would be those fatty acids possessing multiple double bond systems as do the essential fatty acids, but not in the 1:4 cis configuration. Such acids would be those containing conjugated double bonds or double bonds in the 1:4 trans arrangement. R. T. Holman (*Proc. Soc. Exp. Biol. & Med.* 76:100, 1951) and Homan and S. I. Greenberg (*Arch. Biochem. Biophys.* 49:49, 1954), reported that the supplementation of a fat-free diet with conjugated "linoleic" acid resulted in lower weight gains and greater deficiency symptoms in rats than in control animals without any supplementation. D. Melnick and H. J. Deuel, Jr. (*J. Am. Oil Chem. Soc.* 31:63, 1954; *Nutrition Reviews* 13:53, 1955), on the other hand, reported that although some of the trans positional isomers of oleic acid could not replace biotin for *L. arabinosus* and showed some toxicity, they were not inhibitors of oleic acid activity. Similarly these authors found no evidence for EFA inhibition for rats from hydrogenated oils.

Recently, Holman and E. Aaes-Jorgensen (*Proc. Soc. Exp. Biol. & Med.* 93:175, 1956) tested the effect of trans isomers of linoleic acid upon the development of EFA deficiency in rats. Male rats which had been on a fat-free diet containing 1 per cent each of cholesterol and hydrogenated coconut oil to accelerate the onset of deficiency symptoms were, after three months, divided into nine groups receiving the following supplements: ethyl elaidate, ethyl linoleaidate (with or without linseed oil), methyl cis, trans linoleate (with or without linseed oil), methyl linolenate, ethyl linoleate, linseed oil or no supplements. After three weeks of the supplements (0.1 gm. per day), the rats were killed and the severity of the deficiency state, if any, was assessed by examination for skin symptoms, weight gain, carcass fatty acids, condition of spermatogenic tissue and deposition of dietary trans acids. Since each group contained only two rats, differences had to be very marked to be significant. The lowest weight gains were recorded with the cis, trans linoleate and the linolelaidate supplements although the other trans acid, ethyl elaidate, produced a weight increase somewhat greater than that for the fat-free diet. Linseed oil counteracted the effect of the trans dienes and gave weight gains similar to those for the other supplements.

It is difficult to say, on the basis of these experiments, whether the trans acids indeed acted as competitive inhibitors of EFA action. That trans monoenoic esters from hydrogenation of triolein do not, was recently confirmed by R. B. Alfin-Slater et al. (*Ibid.* 95:521, 1957) in very similar experiments. However, it was not to be expected that the trans monoenoic acids would act in this manner, and the cis, trans and trans, trans dienoic esters apparently acted as inhibitors in that they increased the requirement of EFA and aggravated the deficiency symptoms resulting from a fat-deficient diet.

From *Nutrition Reviews*, Vol. 16, No. 2, pp. 59-60, 1958.

Role of the Pituitary in the Response of Rats to Tolbutamide

W. E. Dulin, Ph.D., and W. L. Miller, Jr., Ph.D., Kalamazoo

The most acceptable explanation of the major mechanism of action of tolbutamide (1-butyl-3-*p*-tolylsulfonylurea) in depression of blood sugar is that it stimulates insulin secretion by the β cells of the pancreas.[1-3] There have been several differences found, however, between the effects of insulin and tolbutamide; one of these is that whereas insulin is more effective in hypophysectomized animals, the hypoglycemic effect of tolbutamide is no greater after the removal of the pituitary.[4,5] These observations could be interpreted to mean that tolbutamide produces its hypoglycemic activity by an action other than stimulation of insulin secretion. It should be emphasized, however, that many other factors could be involved in sensitivity of various animal preparations to tolbutamide and should be elucidated before such an interpretation could be considered valid. For example, it has been suggested that the sulfonylureas can stimulate the adrenal medulla in the intact animal, and it has been shown that the secretions of this gland can counteract the effects of these drugs on blood sugar.[6,7] Since the hypophysectomized rat also possesses the adrenal medulla, this organ could be involved in the response of this animal preparation to the hypoglycemic activity of tolbutamide. Another factor which should be considered is that the metabolism of the hypophysectomized rat is altered,[8-10] and it is possible that synthesis of insulin could be adversely affected and result in a lesser amount released from the β cells following tolbutamide treatment. The results of experiments designed to demonstrate differences in insulin content and secretion of insulin by the pancreas of intact and hypophysectomized rats have been conflicting.[11-13]

A study was undertaken, therefore, to analyze the possible factors involved in the failure of hypophysectomy to render rats more sensitive to the hypoglycemic activity of tolbutamide. These studies included an analysis of the effects of tolbutamide on blood sugars at various intervals following hypophysectomy, the effects of removal of the adrenal cortex and/or the adrenal medulla on the response of the hypophysectomized animals, and a comparison of the ability of tolbutamide to mobilize pancreatic insulin in the hypophysectomized and intact rat.

Presented at the Eighteenth Annual Meeting of the American Diabetes Association in San Francisco on June 22, 1958.

From the Research Laboratories, The Upjohn Company.

METHODS AND RESULTS

Comparative effects of tolbutamide on the blood sugar response of intact and hypophysectomized animals.

All animals used in these experiments were obtained from the Upjohn colony (Sprague-Dawley ancestry). Intact rats were used at a body weight of 140-160 gm. The hypophysectomized rats were operated at a body weight of 140-160 gm. and were maintained on ad libitum diet of Purina laboratory chow and tap water. Tolbutamide was administered in CMC vehicle by stomach tube following an eighteen-hour fast and blood sugar was determined by the Nelson procedure[14] on blood taken from the jugular vein two hours following treatment while the animals were under barbiturate anesthesia. Control animals received only the vehicle. The hypophysectomized animals were tested for tolbutamide sensitivity three days, one, two, and four weeks following the operation; the body weights at the time of the experiments were 130-135, 125, 125, 110-125 gm., respectively.

The minimum effective dose required to cause a depression of blood sugar in the intact animal was 50 mg./kg. (figure 1) while the maximum response was obtained with both 100 and 200 mg./kg. The four-week postoperative hypophysectomized animal was less sensitive to tolbutamide than the intact animal since 50 mg./kg. produced only a slight depression of blood sugar and the 100 mg./kg. did not produce the maximum effect (figure 1). The one-week and two-week postoperative rats were slightly more sensitive to tolbutamide since 50 mg./kg. produced a more marked depression of blood sugar than observed in the intact animal and almost as much change as the higher doses. It was also observed that an occasional animal receiving the high dose of tolbutamide in these two groups would

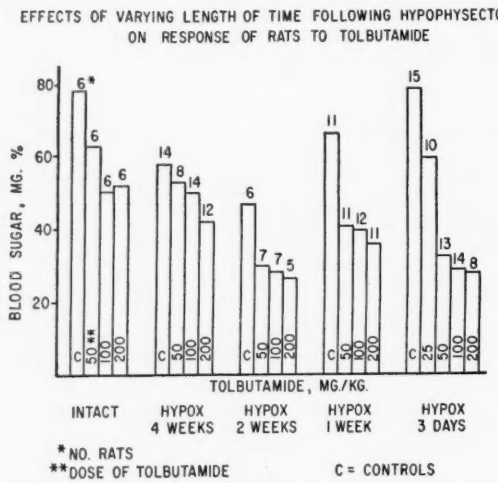


FIGURE 1

be convulsing at the end of the experimental period. In the case of the three-day postoperative animal there was a considerable increase in sensitivity over that observed for the intact rat since 25 mg./kg. caused a significant depression of blood sugar while the 50, 100 and 200 mg./kg. doses produced a greater fall of blood sugar than observed with similar doses in the intact animal.

Effects of tolbutamide or insulin on blood sugars of hypophysectomized-adrenalectomized rats and the effects of tolbutamide on the hypophysectomized-adrenal demedullated rat.

The hypophysectomized-adrenalectomized animals were hypophysectomized at a body weight of 140-160 gm. and two weeks later the adrenals were removed. In the case of the hypophysectomized-adrenal demedullated animals, the adrenal demedullation was done at a body weight of 80-90 gm. and the hypophysectomies were carried out four weeks following the first operation in order to allow time for regeneration of the adrenal cortex prior to removal of the pituitary. The former animals were given Purina laboratory chow and tap water ad libitum until adrenalectomy was done, after which they were maintained on 1 per cent sodium chloride drinking fluid. The hypophysectomized-adrenal demedullated animals were maintained on regular food and 1 per cent sodium chloride for one week following adrenal demedullation after which tap water was substituted for the saline for the remainder of the pretreatment period. At the time of tolbutamide treatment the body weights of the hypophysectomized-adrenalectomized animals averaged 120 gm. while those of the hypophysectomized-adrenal demedullated animals averaged 170 gm. Tolbutamide was administered by stomach tube following an eighteen-hour fast four weeks after hypophysectomy. Blood sugars were determined by the Nelson procedure on blood obtained from the jugular vein while the animals were under barbiturate anesthesia. The insulin was injected subcutaneously in saline with the *p*H adjusted to approximately four by addition of 1 N hydrochloric acid.

The response of the hypophysectomized-adrenalectomized rat was greater than that occurring in the hypophysectomized or intact animal (figures 1 and 2) since as little as 10 mg./kg. of tolbutamide produced a significant depression of blood sugar (figure 2). It is also seen that this animal preparation is no more sensitive to insulin than the hypophysectomized rat (figure 2). The hypophysectomized-adrenal demedullated animal was found to be equally as sensitive to the hypoglycogenic activity of tolbutamide as the hypophysectomized-adrenalectomized rat, since a marked depression of blood sugar was also obtained with as little as 10 mg./kg. This can be contrasted to the four-week postoperative hypophysectomized rat in which 100 mg./kg. was required to produce a significant depression of blood sugar.

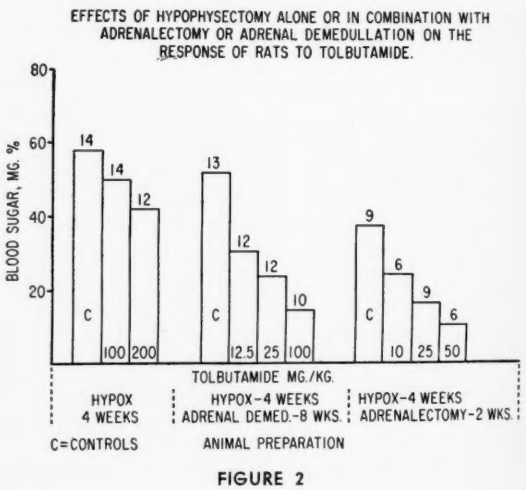


FIGURE 2

Effects of tolbutamide on the extractable insulin of the pancreas of intact and four-week-postoperative hypophysectomized rats.

Animals used in these experiments were obtained from the Upjohn colony. The intact rats weighed 140-160 gm. at the time of the experiment. The hypophysectomized rats weighed 130-150 gm. at the time of the

operation and were kept four weeks prior to being sacrificed at which time they weighed 110-130 gm. Any of the operated animals which gained weight during the four-week period were discarded prior to the experiment. In all of these experiments the animals were fasted eighteen hours prior to autopsy and tolbutamide was given by stomach tube. Prior to the removal of the pancreas, jugular vein blood was obtained for sugar determinations while the animals were under barbiturate anesthesia.

After removal, the pancreata were immediately frozen in a dish surrounded by acetone and dry ice. In all cases the entire pancreatic area was stripped from the duodenum and usually contained mesentery, lymph nodes and fat tissue. The frozen tissue was pooled into groups and immediately homogenized in 0.9 per cent aqueous sodium chloride (8-10 pancreata per 100 ml. saline) for thirty seconds in a chilled Waring Blendor. The homogenate was transferred to a round-bottomed flask, shell frozen and lyophilized overnight.

The insulin activity was extracted from the dry residue by a procedure[15] modified from that originally published.[16] A typical extraction was as follows: The lyophilized tissue which resulted from 8-10 pancreata was extracted with 50 ml. of reagent 1 (ethanol-ethyl ether, 3:1), for ten minutes by a reciprocal shaker set for about three oscillations per second. The mixture was centrifuged at 2,000 RPM for ten minutes and the supernatant discarded. The tissue remaining in the centrifuge tube was transferred back to the original extraction flask, and the entire sample was extracted again with 20 ml. of reagent 1 for ten minutes, after which the mixture was again centrifuged and the supernatant discarded. The insulin activity was then extracted from the residue by two successive extractions with 15 ml. of reagent 2 (31.5 ml. of water, 110 ml. 95 per cent ethanol and 1 ml. of 12 N HCl). After centrifugation, the combined supernatants were stored at 0° C. for thirty minutes and then centrifuged again in the cold at 3,000-4,000 RPM. Any precipitate present was then discarded. The supernatant was transferred to a 24/32 Visking casing for sixteen hours of dialysis at 25° C. against running de-ionized water. The content of the dialysis bag was centrifuged, and the clear supernatant lyophilized to dryness. The resulting powder was taken up in an adequate amount of sterile 0.9 per cent sodium chloride solution, adjusted to *p*H of approximately four and injected for assay.

The extracts were assayed for insulin-like activity by their ability to lower the blood sugars of glucose-primed, seven-day-postoperative adrenalectomized rats which had been fasted for eighteen hours. The glucose priming was carried out by injection of 100 mg. of glucose subcutaneously into each animal immediately prior to the injection of the test material or insulin. Blood sugars were determined by the Nelson method on jugular vein blood obtained while the animals were under barbiturate anesthesia two hours following subcutaneous injection of the insulin or the unknown. The assay animals weighed an average of 150 ± 10 gm. and in all assays the unknowns were given at two doses and the insulin* at two or three doses. Each assay consisted of five to seven assay animals per dosage group, and in most cases the experiments, as well as the assays, were repeated at least twice.

EFFECT OF HYPOPHYSECTOMY ALONE AND IN COMBINATION WITH ADRENALECTOMY ON RESPONSE OF RATS TO INSULIN

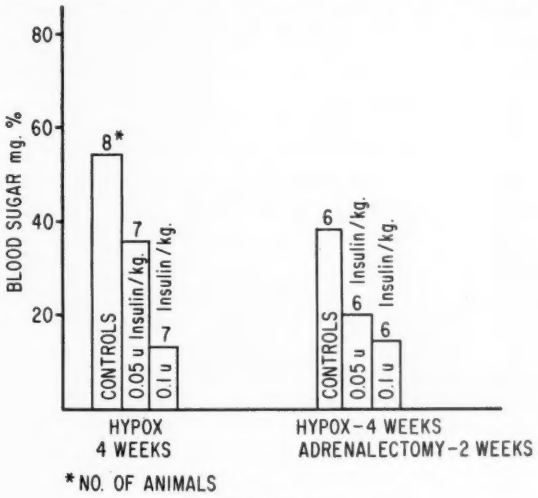


FIGURE 3

A typical experiment showing the results of this assay method is summarized in table 1 in order to indicate the type of experimental results obtained. In all cases the potencies of the unknowns were calculated statistically by the Irwin method.[17] It is seen from these results that an acceptable dose-response is obtained following administration of various doses of insulin or test extract to the glucose-primed adrenalectomized rat.

A comparison of the insulin content of intact and hypophysectomized rats is shown in table 2. The average of 0.60 units of insulin activity per pancreas of the intact animal is greater than 0.33 units per rat obtained

* The insulin used as a standard was crystalline zinc insulin which possessed 24.6 units/mg. (Lot No. 665598). It was kindly supplied by Dr. Otto Behrens of Eli Lilly and Company, Indianapolis, Indiana.

TABLE 1

Typical assay of extractable pancreatic insulin from control and tolbutamide-treated four-week postoperative hypophysectomized rats four hours following treatment

A. Data from rats donating pancreas

No.	Treatment	Blood sugar (mg. per cent)	Final volume containing pancreatic extract
12	Control	54	20 ml.
14	300 mg./kg. of tolbutamide	33	20 ml.

B. Results of assay of pancreatic extracts from donor rats shown in table 1A

No.	Treatment	Dose	Blood sugar (mg. per cent)	Insulin activity (units per pancreas)
		Assay No. 1		
5	Controls	—	87	—
5	Insulin	.02 units	60	
5	Insulin	.04 units	27	
5	Pancreas extract from control hypox rats	.15 cc.	45	.26
5	Pancreas extract from control hypox rats	.30 cc.	27	
5	Pancreas extract from tolbutamide-treated hypox rats	.15 cc.	50	.21
5	Pancreas extract from tolbutamide-treated hypox rats	.30 cc.	32	
		Assay No. 2		
6	Controls	—	72	
6	Insulin	.015 units	53	
6	Insulin	.030 units	41	
6	Insulin	.045 units	26	
6	Pancreas extract from control hypox rats	.10 cc.	47	.28
6	Pancreas extract from control hypox rats	.20 cc.	39	
6	Pancreas extract from tolbutamide-treated hypox rats	.10 cc.	56	.19
6	Pancreas extract from tolbutamide-treated hypox rats	.20 cc.	44	

from the pancreas of the hypophysectomized animal. It is interesting to note that tolbutamide does not produce any significant depression in the extractable insulin of the intact animal's pancreas until four hours following treatment, at which time there is an equivalent of 0.20 units per rat less in the treated animals. In the case of hypophysectomized animals, only a very slight decrease (0.075 units per rat) was obtained in the extractable insulin content four hours following tolbutamide administration, while there was no depression two hours after treatment (figure 4).

DISCUSSION

The failure of the hypophysectomized rat to exhibit increased sensitivity to tolbutamide while this animal preparation does show a marked sensitivity to insulin has been confirmed. These results could be interpreted in several ways: (1) counter-regulatory mechanisms are

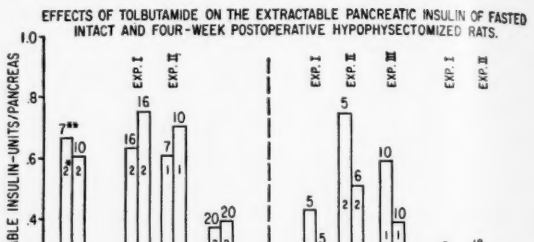


FIGURE 4

present in both animal preparations which may be stimulated by tolbutamide and which can counteract the hypoglycemic effects; (2) tolbutamide does not act by stimulation of insulin secretion by the β cells of the pancreas; (3) there is less insulin released from the pancreas of the hypophysectomized rat compared to the amount released from the pancreas of the intact animal following sulfonylurea treatment; or (4) a combination of two or more of these factors may be involved in failure of the operated animal to become more sensitive to hypoglycemic activity of tolbutamide.

It should be pointed out that a difference was found in the sensitivity of the hypophysectomized rat depending on the length of time following the operation prior to treatment with tolbutamide. However, the observation showing that the blood sugar response plateaued in all of the hypophysectomized rats as well as the intact animals suggests that tolbutamide is capable of stimulating some of the same counter-regulatory mechanisms in both animal preparations. In contrast, this plateauing of blood sugar response does not appear to be the case with insulin since intact rats as well as hypophysectomized rats can be made extremely hypoglycemic with this drug.[18,19] The organ which is probably responsible for the leveling off of the blood sugar response of both the intact and hypophysectomized rat was the adrenal medulla, since removal of either the entire adrenal or only the adrenal medulla causes the hypophysectomized rat to become considerably more sensitive to the hypoglycemic activity of tolbutamide. This has also been found to occur in the nonhypophysectomized animal.[9] The observation showing that the hypophysectomized rat and the hypophysectomized-adrenalectomized rat are equally as sensitive to insulin

indicates that insulin at doses used in these studies apparently did not stimulate the adrenal medulla to counteract its hypoglycemic activity in the hypophysectomized animal. These results further suggest that decrease in blood sugar per se is not the responsible factor for stimulation of the adrenal medulla and indicate tolbutamide may have a direct effect on this organ.

The data showing less extractable insulin (or insulin-like activity) in the pancreas of the four-week-postoperative hypophysectomized rat indicates that one reason for the lack of sensitivity of this operated animal to tolbutamide is a decrease in available mobile insulin. This hypothesis was supported by the fact that only a total of 0.075 units of insulin per animal over a period of four hours disappeared from the pancreas of the four-week-postoperative hypophysectomized rat under tolbutamide stimulation as compared to approximately 0.20 units of insulin per animal in the case of the unoperated rat. These effects on the insulin content of the pancreas in the intact rat agree with the observations of others on the dog and calf.[20,21] When this is considered along with the observations suggesting that tolbutamide may cause a stimulation of the adrenal medulla, and since a published report has shown that epinephrine can counteract the hypoglycemic effects of tolbutamide,[7] one must conclude that a combination of two factors can account for the failure of the hypophysectomized animal to show heightened sensitivity to the hypoglycemic activity of this sulfonylurea. These are: (1) that hypophysectomy caused a decrease in available insulin which can be mobilized under stimulation of tolbutamide, and (2) that tolbutamide may stimulate the adrenal medulla of both intact and hypophysectomized animals to secrete epinephrine which can counteract the hypoglycemic activity of the released insulin. Therefore, the earlier reports showing that hypophysectomized and intact animals are equally as sensitive to tolbutamide while the hypophysectomized animal exhibited an increased sensitivity to insulin should not be interpreted to mean that the principal mechanism of action of tolbutamide in lowering blood sugar was not mediated through release of insulin by β cells.

It should be noted that it takes a total of four hours post-treatment for a measurable drop in pancreatic insulin content in the intact rat, while the depression of blood sugar reaches a maximum in about two hours.[23] The finding of von Holt et al.[22] that there is an increase in plasma insulin concentration two hours following sulfonylurea treatment, while our results indicated that the insulin content of the pancreas was not measurably decreased for four hours, indicates that there may be increased synthesis of insulin by the β cells following treatment and it takes two to four hours for the release of insulin to overcome the rate of synthesis. It is obvious that to get a true rate of insulin release, it would be ideal to measure the changes in insulin concentration of both the pancreas and pancreatic vein blood continuously. Nevertheless, since the pancreas of the hypophysectomized rat contained less insulin than that of the intact animal and since following tolbutamide treatment the total change in pancreatic insulin content was less in the hypophysectomized animal, it is concluded that under the influence of tolbutamide the β cells of the hypophysectomized rat actually secreted less insulin than those of the intact animal.

SUMMARY

Four-week-postoperative hypophysectomized rats were found to be slightly less sensitive to the hypoglycemic effects of tolbutamide than the intact animal. One or two-week-postoperative rats were equally or slightly more responsive, while the three-day-postoperative animal was considerably more sensitive to tolbutamide than the intact animal. When the adrenal medulla and/or the adrenal cortex were removed from the hypophysectomized animals, they became extremely sensitive to the hypoglycemic activity of the drug. In contrast, adrenalectomy of hypophysectomized rats did not increase the sensitivity to the hypoglycemia induced by injected insulin.

The extractable insulin content of the pancreas of the four-week-postoperative rat was approximately one half of that present in the pancreas of the intact rat. The results of these experiments were interpreted to mean that the difference in sensitivity of the four-week postoperative hypophysectomized rat to insulin and tolbutamide does not imply that tolbutamide does not cause a stimulation of insulin secretion but is a result of a depression of insulin content of the pancreas in the operated animal and consequently a decrease in the amount of insulin which can be mobilized under tolbutamide stimulation. In addition, the hypophysectomized animal possesses one of the same counter-regulatory mechanisms as the intact animal—the adrenal medulla—which can be stimulated by tolbutamide to release epinephrine. It is thought that the epinephrine, therefore, counteracts the activity of the decreased quantity of insulin released from the pancreas of the hypophysectomized animals treated with tolbutamide.

SUMMARIO IN INTERLINGUA

Le Rolo Del Glandula Pituitari In Le Responsa De Rattos A Administrationes De Tolbutamido

Esseva trovate que rattos, quatro septimanas post hypophysectomia, esseva levemente minus sensibile al

TABLE 2

Summary of results of experiments measuring pancreatic insulin content of untreated intact and hypophysectomized rats

Animal preparation	Intact		Hypophysectomized
Calculated extractable insulin units per pancreas	*.58 (10)	.63 (16)	*.38 (23)
	†.42 (5)	.58 (24)	†.28 (12)
	.74 (5)	.66 (7)	.27 (6)
	.60 (7)		.37 (20)
	Ave. = .60		Ave. = .33

( ) = No. of pancreata extracted in each experiment.
*Done at same time. †Done at same time.

effecto hypoglycemic de tolbutamido que animales intacte. Un o duo septimanas post le operation, le rattos habeva le mesme sensibilitate pro tolbutamido como le rattos intacte, excepte in alicun casos quando lor sensibilitate esseva levemente plus grande. Tres dies post le operation le sensibilitate esseva considerabilemente plus grande. Quando le medulla adrenal e/o le cortice adrenal esseva excidite ab le animales previemente hypophysectomisate, illes deveniva extrememente sensibile al effecto hypoglycemic del droga. Del altere latere, adrenalectomia in rattos hypophysectomisate non augmentava lor sensibilitate al effecto hypoglycemic de injectiones de insulina.

Le inextrahibile contento de insulina in le pancreas de rattos quatro septimanas post hypophysectomia esseva circa un medietate del correspondente contento in le pancreas de animales intacte. Le resultatos de iste experimentos esseva interpretate como indication que le differentia inter le sensibilitate pro insulina e le sensibilitate pro tolbutamido que se observa in rattos hypophysectomisate quatro septimanas post le operation non justifica le conclusion que tolbutamido non causa un stimulation del secretion de insulina. Il pare plus tosto que illo es le resultato de un depression del contento de insulina in le pancreas del operate animales, lo que significa que solmente un reducite quantitate de insulina es disponibile pro esser mobilisate per le stimulo de tolbutamido. In plus, le hypophysectomisate animal possede ancora un del mechanismos contra-regulatori que es presente in le animal intacte, i.e. le medulla adrenal, que pote esser stimulate per tolbutamido a discargar epinephrina. Es opinate que le epinephrina age contra le activitate del reducite quantitate de insulina discargate per le pancreas de animales hypophysectomisate e tractate con tolbutamido.

ACKNOWLEDGMENT

The authors acknowledge technical assistance of M. C. Blanks, I. C. Kent, R. Lapsa and J. Krake.

REFERENCES

[1] Loubatieres, A.: Analyse du mecanisme de l'action hypoglycemiante du *p*-aminobenzene-sulfamido-thiodiazol. Compt. rend. soc. biol. *138*:766-67, 1944.

[2] Pozza, G., Galansino, G., and Foa, P. P.: Insulin secretion following carbutamide injections into normal dogs. Proc. Soc. Exp. Biol. & Med. *93*:539-42, 1956.

[3] Loubatieres, A.: The mechanism of action of the hypoglycemic sulfonamides: A concept based on investigations in animals and human beings. Annals of the N. Y. Acad. of Sciences *71*:192-206, 1957.

[4] Houssay, B. A., and Penhos, J. C.: Action of the hypoglycemic sulfonyl compounds in hypophysectomized, adrenalectomized and depancreatized animals. Metabolism *5*:727-32.

[5] Wall, J. S., Steele, R., DeBodo, R. C., and Altszuler, N.: Mechanism of insulin hypersensitivity in the hypophysectomized dog. Am. J. Physiol. *189*:51-56, 1957.

[6] Lang, S., and Sherry, S.: Some effects of Orinase in the rat. Metabolism *5*:733-38, 1956.

[7] Fajans, S. S., Louis, L. H., Seltzer, H. S., Johnson, R. D., Gittler, R. D., Hennes, A. R., Wajchenberg, B. L., Ackerman, I. P., and Conn, J. W.: Metabolic effects of arylsulfonylurea compounds in normal men and diabetic subjects. Metabolism *5*:820-39, 1956.

[8] Houssay, B. A.: Advancement of knowledge of the role of the hypophysis in carbohydrate metabolism during the last 25 years. Endocrinology *30*:884-97, 1942.

[9] Samuels, L. T., Reinecke, R. M., and Bauman, R.: Growth and metabolism of young hypophysectomized rats fed by stomach tube. Endocrinology *33*:87-95, 1943.

[10] Samuels, L. T.: The relation of the anterior pituitary hormones to nutrition. Recent Progress in Hormone Research *1*:147-76, 1947.

[11] Haist, R. E.: The pituitary and the insulin content of the pancreas. J. Physiol. *98*:419-23, 1940.

[12] Fraenkel-Conrat, H. L., Herring, V. V., Simpson, M. E., Evans, H. M.: Effect of purified pituitary preparation on the insulin content of the rat's pancreas. Am. J. Physiol. *135*:404.

[13] Randle, P. J.: Pituitary growth hormone and blood insulin activity. Ciba Foundation Colloquia on Endocrinology *9*:35-54.

[14] Nelson, N.: Photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. *153*:375-80.

[15] Simpson, M. V.: Personal communication.

[16] Light, A., and Simpson, M. V.: Studies on the biosynthesis of insulin. Biochim. et Biophys. Acta. *20*:251-61, 1956.

[17] Irwin, J. O.: Statistical methods applied to biological assays. Royal Stat. Soc. Supplement *IV*:1-60, 1937.

[18] Glickman, N., and Gellhorn, E.: The effect of oxygen deficiency on the sensitivity of rats to insulin. Am. J. Physiol. *121*:358-63, 1938.

[19] Gellhorn, E., Feldman, J., and Allen, A.: Assay of insulin on hypophysectomized, adrenal demedullated and hypophysectomized-adreno-demedullated rats. Endocrin. *29*:137-40, 1941.

[20] Root, M.: Effect of carbutamide on the insulin content of the dog pancreas. Diabetes *6*:12-16, 1957.

[21] Pfeiffer, E. F., Steigerwald, H., Sandritter, W., Bander, A., Beaker, U., and Retiene, K.: Comparative studies of the morphology and hormonal content of calf's pancreas after administration of D-860. Deutsche med. Wchschr. *82*:147-62, 1957.

[22] Holt, C., von; Holt, L., von; Kracht, B.; Kroner, B.; and Kuhnau, J.: Carbutamide and plasma insulin activity. Science *125*:735, 1957.

[23] Dulin, W. E., Morley, E. H., and Nezamis, J. E.: Role of the adrenal in response of rats to Orinase. Proc. Soc. Exp. Biol. & Med. *93*:132-36, 1956.

Effects of Growth Hormone on the Transport of Lipids in Blood

G. F. Wilgram, M.D., Ph.D., J. Campbell, Ph.D., Lena Lewis, Ph.D., and Jean Patterson, Ph.D., Toronto

The view is widely held that the forms in which lipids are carried in the blood may be of importance in atherosclerosis and related disorders.[1] A comparative study of lipid transport under the conditions of mobilization of fat from the body depots and of absorption of fat from the intestine was therefore undertaken. Mobilization was induced by fasting and by the administration of purified growth hormone. Fat absorption, with ensuing movement of lipid towards the depots, was obtained by feeding a fat meal. Lipid transport was also studied under conditions of increased fat utilization in the prediabetic state caused by repeated injections of growth hormone in regularly fed dogs. The results demonstrate marked differences in the amounts and characteristics of the lipids transported in blood under these conditions.

METHODS, MATERIALS AND EXPERIMENTAL PROCEDURES

Adult dogs of either sex were kept in metabolism cages in air-conditioned rooms. Venous blood samples were collected with heparin as anticoagulant for determinations of sugar, erythrocyte sedimentation rate and volume per cent of corpuscles and of plasma by methods given previously.[2] Blood samples were allowed to clot for one hour at 4° C. The optical density and the unesterified fatty acids (UFA) of the sera were determined according to Grossman, Stadler, Cushing and Palm.[3] Values for blood lipids were obtained by a slight modification of Bloor's alcohol-ether extraction method.[4] The alcohol-ether extracts were taken to dryness in vacuo (water-bath 45°); lipids in the residue were taken up in 3:1 (v/v) petroleum ether (b.p. 40-60°)—chloroform. Cholesterol was determined on aliquots of the petroleum ether-chloroform solutions by the method of Sperry and Webb.[5] Phospholipid P was determined by King's method[6] and phospholipid was calculated using the factor 25.0 x P. Total lipids were determined gravimetrically on aliquots of the petroleum ether-chloroform mixture after evaporation of the solution. No total fatty acid and cholesterol ester determinations were carried out which would allow for accurate calculation of serum triglycerides. A rough estimation, however, of the serum triglyceride level may be arrived at by subtracting the values for phospholipids and total cholesterol from the values for total lipids.[7-9] Approximately two thirds of this difference may be regarded as "neutral fat," the remaining one third being due to cholesterol ester fatty acid and unesterified fatty acid (UFA or NEFA). The serum lipoprotein fractions were estimated by the ultracentrifugal technic of Lewis, Green and Page.[10] The —S 20-40 and —S 1-20 fractions are referred to as β and α lipoproteins, respectively. The estimation of the lipoproteins at d 1.21 does not permit measurement of the extremely low density chylomicrons. For a determination of this component optical density has been used. The amount of blood required for all these determinations was approximately 35 ml. These large withdrawals of blood were tolerated without apparent changes. The times of the blood withdrawals are indicated in figure 1. In the experiment on fasting (figure 1, column 1) the first blood sample was withdrawn six hours postprandially; in the experiment on fat feeding prior to the test meal, which was given forty-two hours after the last food intake (column 2). When growth hormone was injected into fasting animals, the first blood sample was taken forty-two hours postprandially as well (figure 1, column 3). In the dogs fed twice daily and injected with growth hormone (column 4) the blood samples were taken in the "fasting" state prior to their morning meal, that is, eighteen hours after their last food intake on the previous day. *No postprandial* blood samples were taken from these growth-hormone injected, fed dogs. The experimental periods were separated by intervals of two to three weeks.

Previous to, and during the intervals between the

From the Banting and Best Department of Medical Research and Department of Physiology, University of Toronto, and The Research Division of the Cleveland Clinic Foundation, Cleveland, Ohio.

Presented before the Canadian Federation of Biological Societies, Kingston, Ontario, June, 1958.

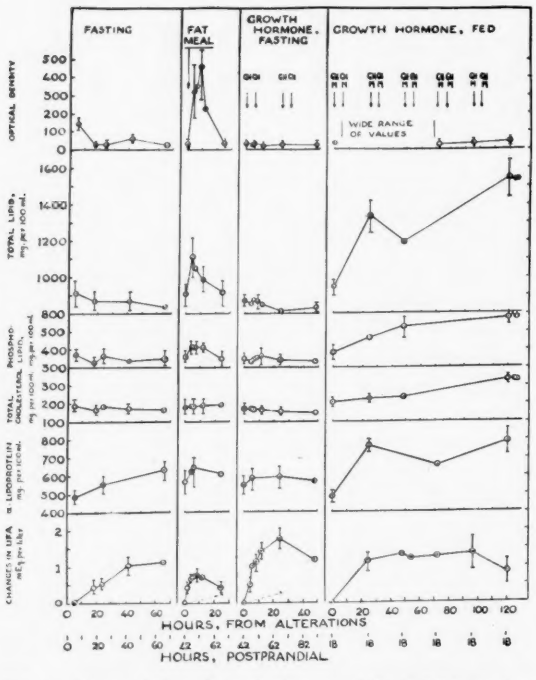


FIG. 1. Mean values and standard errors of blood lipids of nine dogs. G. H. indicates the injection of growth hormone, M the administration of a meal. The broken lines under columns "Fat Meal" and "Growth Hormone, Fasting" indicate the rises in UFA due to fasting alone. Note that the UFA are expressed as changes from the initial value.

experiments, the dogs were given meals, at 10 a.m. and 4 p.m., of horse meat in the amount of 1,430 calories per square meter of body surface per day. At all times water was available ad libitum. In the experiment on fat absorption the dogs were fasted for a day and on the following day at 10 a.m. (forty-two hours postprandial) were given the test meal of 10 gm. fat per kilogram of body weight, which was usually consumed within half an hour. In the case of two dogs (774 and 860) it was mixed with about 100 gm. of horse meat to increase its palatability. The fat fed was either lard, to dogs 471, 859 and 860, or fat rendered from dogs' adipose tissues, to dogs 771 and 774. In the experiment on fat mobilization the dogs were fasted for a day and on the following day were given subcutaneous injections of growth hormone at 10 a.m. (forty-two hours postprandial) and 4 p.m. The dosage of growth hormone, per kilogram of body weight per day, was 10 mg. to dogs 771 and 774, and 3 mg. to the others. In the feeding experiments 1.5 mg. of growth hormone (total dose daily 3 mg.) per kilogram of body weight was given subcutaneously at the time of feeding for five days. The purified, crystalline growth hormone (Campbell and Davidson),[11] Connaught Medical Research Laboratories, Lot 100, was generously provided jointly by the National Research Council of Canada, The National Cancer Institute and the Department of Health and Welfare. It was dissolved each day in saline at *p*H 8, 10 mg. per milliliter, for subcutaneous injection.

RESULTS

The mean results for the nine dogs used in these experiments are summarized in figure 1, and standard errors are indicated. As each animal served as its own control the consecutive values in each dog are more meaningful than is indicated by the standard errors. However, because of the mass of data, the individual values are presented for only one typical animal, dog 774 (table 1).

In the experiment on fasting animals, some effect of the last meal on optical density was evident at six hours, but from eighteen to sixty-six hours postprandial the serum remained clear. Following the fat meal the optical density rose sharply to a peak at eight hours; was still high at twelve hours but not at twenty-four hours. Much variation occurred in this response, both with respect to the time at which the peak occurred and to its height. When growth hormone was administered to the fasting dogs the optical density remained low. When growth hormone was given repeatedly for five days to fed dogs, the serum samples, taken eighteen hours postprandially, usually showed opacity within the first two days of injection, but by the third day had cleared. (Opacity of the serum during the first few days of injection of growth hormone has been noted in several other fed dogs in this laboratory. The serum then became clear despite repeated injections.)

The total lipids of the serum remained low under the conditions of fasting, and during the administration of growth hormone in the fasting state. They rose following the fat meal. The total lipids increased to the greatest extent when the dogs were fed and injected repeatedly with growth hormone; one day after the first injection the increase in lipids of the serum taken eighteen hours postprandially were definite, and after five days were still higher. The phospholipids of the serum did not change appreciably either during fasting, after the fat meal or when growth hormone was given during a period of fasting. However, a significant increase occurred when the dogs were fed and given growth hormone twice daily for five days. Likewise, the total cholesterol of the serum increased when the

TABLE 1

Serum lipids of a typically behaving dog (774) under various conditions

This dog was studied under the following conditions: (a) Fasting for 66 hr. (b) Given 42 hr. after the last food intake, a fat meal equivalent to 10 gm. per kg. of body weight. (c) Fasted for 42 hr. then given 10 mg. growth hormone per kg. of body weight per day during continued fasting for two days. (d) Regularly fed and injected twice daily with growth hormone, 3 mg. per kg. of body weight per day.

	Fasting					Fat meal					Growth hormone and fasting						Growth hormone and fed					
Lipid fraction Pp*	6	18	24	42	66	0	4	6	8	24	42	46	48	54	66	90	18	18	18	18	18	18
of serum Pi†											0	4	6	12	24	48	0	24	48	72	96	120
Optical density‡	155	29	29	38	22	25	495	393	260	28	39	24	49		32	22	19	160	112	25	37	32
Total lipids mg./ 100 ml.	1,009	992	816	775	814	988	1,259	1,181			889		876		809	830	1,040	1,205	1,370			1,623
Phospho-lipid mg./ 100 ml.	416	363	375	323	338	441	486	473			375		367		349	350	397	417	479			641
Total choles-terol mg./ 100 ml.	217	185	200	182	160	224	231	223			170		163		154	150	204	209	217			369
β-lipo-proteins (-S 20-40) mg./100 ml.		130	107	44	38	197	137	124			44		71		59	71	130	240				294
α-lipo proteins (-S 1-20) mg./100 ml.		480	527	535	640	527	564	633			535		595		610	625	420	720				862
Unesteri-fied fatty acids of serum mEq./L.	1.08	1.82	2.06	2.68	2.54	1.24	1.54	1.64	1.34	1.22	1.46	1.86	2.24	2.78	3.14	2.86	0.98	3.04	3.20	3.00	3.40	3.06

*Pp: — hours postprandial.
†Pi: — hours after first injection of growth hormone.
‡Optical density measured at 640 mμ.

dogs were fed and given growth hormone during five days; but did not change appreciably during the other experimental conditions.

The α-lipoprotein fraction (—S 1-20) increased significantly when the dogs were fed and given growth hormone. Likewise the β-lipoprotein fraction (—S 20-40) was affected significantly only under the condition of feeding plus growth hormone.

The UFA of the serum taken eighteen hours after feeding ranged from 0.82 to 1.83 mEq./L. This fraction increased during a fast and reached a plateau at about forty-two hours. The UFA rose slightly and insignificantly following the test meal, which was very high in fat and had very little or no carbohydrate or protein (see Methods). The most marked rise in UFA over the increase due to fasting alone, occurred in the dogs that were fasted and given growth hormone. This increase occurred rapidly with a peak at about twenty-four hours after first injection.* When growth hormone was given regularly with meals, the UFA of the eighteen-hour postprandial serum samples remained elevated during the five days of observation.

Hyperglycemia accompanied by glycosuria occurred in five of the nine dogs when they were fed and given growth hormone for five consecutive days, i.e., in Nos. 521, 647, 774, 471 (slight) and 759 (table 2). Hematocrit readings were obtained for each sample of blood withdrawn. For each of the various conditions to which the dogs were subjected, the final volume per cent of the plasma was either the same as or slightly above the initial value for that period. Confirming previous observations,[2] the erythrocyte sedimentation rate increased in fed dogs given growth hormone. During this period body weight increased by an average 6.9 per cent.

* This finding has been confirmed in man[20] and in monkeys.[21]

TABLE 2

Blood sugar

The dogs and the conditions are as shown in figure 1. The blood sugar values are given as mg. per cent.

		Fasting					Fat meal						Growth hormone, fasting						Growth hormone, fed					
Dog no.	Pp* Pi†	6	18	24	42	66	0	4	6	8	12	24	42 0	46 4	48 6	54 12	66 24	90 48	18 0	6	18 24	18 48	18 72	18 96	18 120
518																			61			78	74	88	52
521																			43			64	86	113	144
647																			55			130	202	112	234
771		69	72	65	54	53	58	61	54		57	49	72	69	72	63	72	89	58	70	72	90	94	83	102
774		63	57	49	49	54	71	67	65		63	60	69	63	61	62	67	84	55	67	81	92	187	210	258
471		74	84	73	62	54	66	90	88		87	81	62	48	37	131	141		74		90				117
859		82	83	73	65	55	65	55	56		70	73	65	74	77	87	63		76		89				103
860		77	66	67	53	49	53	42	44		66	64	46	83	69	72	83		73		75				94
759													35	73	76	88	87		67		170				303

*Pp:—hours postprandial.
†Pi:—hours after first injection of growth hormone.

DISCUSSION

The physiological role of the nonesterified or unesterified fatty acids (NEFA or UFA) of the blood has been discussed by Dole,[12] Gordon,[13] and others. This fraction is derived mainly from the lipids of the adipose tissue and is transported in combination with albumin. Although it constitutes about 5 per cent of the total fatty acids of the plasma[12] the rapidity of its turnover is so great as to suggest that it may be the active form in which lipid is transported from fat depots to the sites of utilization.[12] The UFA are increased in man by fasting,[18] and this occurred also in our dogs. After a continuous rise during the initial stages of fasting a plateau of considerable height was finally reached. The serum remained clear and the total lipids remained low.

Following a fat meal, the absorbed lipids (mostly triglycerides) were transported from the intestine to sites of storage and utilization in a different fashion—chiefly as chylomicra, as indicated by marked opacity of serum. The nonsignificant rise of UFA after this fat meal is probably due to the very high fat and the low carbohydrate content of the diet. Therefore, the expected fall of UFA observed when carbohydrate is fed to the fasting animal[19] may not be anticipated.

Growth hormone causes an increase in liver lipid, ketonemia, and a lowering of the respiratory quotient in the fasting animal. These changes are indicative of fat mobilization.[14] In this state of enhanced transport of fat from body depots the UFA rose sharply beyond the levels due to fasting alone. While a protein-sparing effect is exerted by growth hormone in the fasting animal,[15,16] the rapidity of the rise in UFA would suggest that the fat mobilization is not subsequent to changes in protein metabolism. It is felt, therefore that the rapid rise in UFA is one of the primary effects of growth hormone.*

Much experimental evidence, summarized by de Bodo and Altszuler,[17] indicates that repeated injections of growth hormone in regularly fed animals enhance the mobilization and utilization of fat and the synthesis of protein. Under these conditions a prediabetic or diabetic state is produced in dogs,[14] and in the present experiments, five of the nine dogs given growth hormone for five days exhibited hyperglycemia and glycosuria (table 2). We also found that the lipids of the plasma were altered considerably when dogs were fed and given growth hormone. The turbidity of the serum which occurred within the first two days of injection is unexplained at present, but must be a part of the general, suddenly increased fat mobilization. The serum phospholipids, total cholesterol, the α-lipoproteins and the β-lipoproteins rose higher than at any other time. The total lipids in the "fasting" serum samples of growth-hormone treated animals were higher than after a fatty meal in the same dogs, yet no turbidity was present after five days of injection. Thus, in this state of enhanced mobilization of lipid, the total lipids and the serum triglycerides rose greatly but remained soluble; apparently, the rise in phospholipids, reflected by rises in the lipopro-

* This view is in agreement with results obtained by F. L. Engel, who used growth hormone in in vitro studies.[20]

teins may explain why the triglycerides did not cause any turbidity and the plasma stayed clear in this experimental condition. The significant increase in UFA is another indication for the enhanced mobilization of lipid from the depots in the prediabetic state induced by growth hormone in regularly fed dogs. It was found that this increase in UFA was accompanied by an increase in α and β lipoprotein and that there was a rough correspondence in the degree of change of these three fractions. The explanation of this relationship is not known at present, and may be coincidental. It is also possible that our observations are related to the results of Lindgren and Gofman[18] who found that 6 per cent by weight of the lipid content of the α lipoproteins of human serum are UFA. According to this view, the UFA (NEFA) are thought to be combined in the serum mainly with albumin, but to a lesser extent (one third) also with the α and β-lipoproteins.[19] Thus it appears that one of the many possible roles of the lipoproteins may be to participate in the transport of UFA during their mobilization from the depots.

SUMMARY

Lipid transport during fat absorption. The chief characteristic of serum lipids during fat absorption is a rise in total lipids. This fat load appeared to be carried chiefly as triglyceride in the form of chylomicra, as indicated by an increase in optical density.

Lipid transport during fat mobilization. In the fasting dog given growth hormone, the mobilization of lipid was manifested by a rapid rise in serum UFA, above that due to fasting alone. The serum remained clear and no characteristic changes occurred in its total lipid, phospholipid or total cholesterol. It is felt that the increase in UFA is one of the primary effects of growth hormone.

Lipid transport in the prediabetic state caused by growth hormone. In fed dogs repeatedly injected with growth hormone, a prediabetic state is produced. In this condition lipid mobilization is greatly enhanced. The total lipids rose to high levels, resulting in lipemia without turbidity in the eighteen-hour "fasting" blood samples after the third day. The phospholipids and cholesterol rose markedly reflected by rises in the α and β lipoprotein fractions. The increases in α and β lipoprotein indicate that these fractions may be involved in the transport of large amounts of serum triglycerides in solubilized form in this prediabetic state. A significant, sustained rise in UFA is part in this state of greatly increased fat mobilization. A portion of these elevated UFA is probably carried in the lipoproteins.

SUMMARIO IN INTERLINGUA

Effectos De Hormon De Crescentia Super Le Transporto De Lipidos In Sanguine

Transporto de lipidos durante le absorption de grassia. Le principal characteristica del lipidos seral durante le absorption de grassia es un augmento de lipido total. Il pare que le carga de grassia es portate principalmente como triglycerido in le forma de chylomicrones. Isto es indicate per un augmento del densitate optic.

Transporto de lipidos durante le mobilisation de grassia. In canes in stato jejun que ha recipite hormon de crescentia, le mobilisation de lipido es manifeste in le augmentation rapide del non-esterificate acidos grasse in le sero. Iste augmentation excede le nivello explicabile per le jejuno sol. Le sero remane clar e nulle alteration characteristic occurreva in su lipido total, in su phospholipido, o in su cholesterol total. Es opinate que le augmento del non-esterificate acidos grasse es un del effectos primari del hormon de crescentia.

Transporto de lipidos in le stato prediabetic que es causate per hormon de crescentia. In canes in stato alimentate que ha recipite repetite injectiones de hormon de crescentia, un stato prediabetic es producite. In iste stato, le mobilisation de lipidos es grandemente promovite. Le lipidos total attinge alte nivellos, con le resultato de lipemia sin turbiditate in le specimens de sanguine "jejun" a dece-octo horas a partir del tertie die. Le phospholipidos e le cholesterol es marcatemente augmentate. Isto es reflectite in augmentos del fractiones alpha e beta de lipoproteina. Iste augmentos indica que le fractiones in question es possibilemente concernite in le transporto de grande quantitates de triglyceridos seral in forma solubilisate que occurre in iste stato prediabetic, viste que un significative e continue augmento del non-esterificate acidos grasse es un characteristica del stato de grandemente augmentate mobilisation de grassia. Un portion del augmentate non-esterificate acidos grasse es probabilemente portate in le lipoproteinas.

ACKNOWLEDGMENTS

The authors appreciate Dr. Irvine H. Page's interest in this study. They are grateful to Dr. Charles H. Best for his help and wish to acknowledge the excellent, technical work of Mrs. V. Lazdins.

The investigation was aided by grants from the National Research Council of Canada, the Nutrition Foundation, Inc., and the Life Insurance Medical Research Fund, New York. The lipoprotein part of these studies was financed in part by a grant from the National Heart Institute, United States Public Health Service, Bethesda, Maryland.

REFERENCES

[1] Anfinsen, C. B.: Physiological aspects of lipid transport. Symposium on Atherosclerosis, Washington, D.C., National Academy of Sciences, National Research Council, publication 338, 1954, page 217.

[2] Campbell, J., Hausler, H. R., Munroe, J. S., and Davidson, I. W. F.: Effects of growth hormone in dogs. Endocrinology *53*:134, 1953.

[3] Grossman, M. I., Stadler, J., Cushing, A., and Palm, L.: The relation between lipolysis and deturbidification and re-turbidification in the heparin induced lipemia clearing phenomenon. Medical Nutrition Laboratory, U.S. Army, Denver, Colorado. Report No. 140, 1, 1954.

[4] Bloor, W. R., Pelkan, K. F., and Allen, D. M.: Determination of fatty acids and cholesterol in small amounts of blood plasma. J. Biol. Chem. *52*:191, 1922.

[5] Sperry, W. M., and Webb, M.: A revision of the Schoenheimer-Sperry method for cholesterol determination. J. Biol. Chem. *187*:97, 1950.

[6] King, E. J.: The colorimetric determination of phosphorus. Biochem. J. 26:292, 1932.

[7] Boyd, E. M.: A differential lipid analysis of blood plasma in normal young women by micro-oxydation methods. J. Biol. Chem. *101*:323, 1933.

[8] Boyd, E. M.: The extraction of blood lipids. J. Biol. Chem. *114*:223, 1936.

[9] Page, I. H., Kirk, E., Lewis, W. H., Jr., Thompson, W. R., and Van Slyke, D. D.: Plasma lipids of normal men at different ages. J. Biol. Chem. *111*:613, 1935.

[10] Lewis, L. A., Green, A. A., and Page, I. H.: Ultracentrifugal lipoprotein pattern of serum of normal, hypertensive and hypothyroid animals. Am. J. Physiol. *171*:391, 1952.

[11] Campbell, J., and Davidson, I. W. F.: Preparation and properties of bovine growth hormone. Proc. XIX Internat. Physiol. Congress, page 257, 1953. Therien Frères, Montreal.

[12] Dole, V. P.: Transport of nonesterified fatty acid in plasma. Chemistry of Lipids as Related to Atherosclerosis, Irvine H. Page, editor, Springfield, Illinois, Charles C Thomas, publisher, 1958, page 189.

[13] Gordon, R. S., Jr., and Cherkes, A.: Unesterified fatty acid in human blood plasma. J. Clin. Investigation *35*:206, 1956.

[14] Campbell, J.: Diabetogenic actions of growth hormone. The Hypophyseal Growth Hormone, Nature and Actions. Editors: R. W. Smith, O. H. Gaebler and C. N. H. Long. New York, The Blakiston Division, McGraw-Hill Book Co. Inc., 1955, page 270.

[15] Harrison, H. C., and Long, C. N. H.: Effects of anterior pituitary extracts in the fasted rat. Endocrinology *26*:971, 1940.

[16] Szego, C. M., and White, A.: The influence of growth hormone on fasting metabolism. Endocrinology *44*:150, 1949.

[17] De Bodo, R. C., and Altszuler, N.: The metabolic effects of growth hormone and their physiological significance. Vitamins and Hormones, vol. 15. New York, Academic Press Inc., 1957, page 205.

[18] Lindgren, F. J., and Gofman, J. W.: The role of lipoproteins in coronary disease. Bull. Swiss Acad. Med. Sci. *13*:152.

[19] Lindgren, F. J.: Structure and homogeneity of low density human serum lipoproteins. Conference of the N.Y. Academy of Sciences: The Influence of Hormones on Lipid Metabolism in Relation to Atherosclerosis. April 11, 1958.

[20] Raben, M. S.: Human growth hormone. Presentation before the Laurentian Hormone Conference, Sept. 8, 1958. Recent Prog. Horm. Res. *15*:1959 (in prep.).

[21] Knobil, E., and Greep, R. O.: The physiology of growth hormone with particular reference to its action in the rhesus monkey. Presentation before the Laurentian Hormone Conference, Sept. 7, 1958. Recent Prog. Horm. Res. *15*:1959 (in prep.).

[22] Engel, F. L.: Personal communication, November 1958.

The conversion of certain pentoses to glucose was suggested by the finding that the infusion of 5 to 20 gm. of D-xylose or L-arabinose resulted in a 10 to 30 per cent increase in blood glucose levels. On infusion none of the pentoses affected either the blood lactate or pyruvate concentrations, but all caused a significant decrease in the level of serum inorganic phosphate, this being most marked in the case of D-xylose. In order to obtain information on the fate of the relatively large fraction of D-xylose which did not appear in the urine, two experiments were performed with D-xylose-1-C^{14} in which the disposition of the isotope was determined. Virtually all of the C^{14} recovered from the urine was found in the xylose band on chromatography, less than 0.1 per cent appearing as xylitol or xylonolactone which are two possible metabolites. The expired CO_2 was maximally labeled forty-five minutes after the infusion ended, although the first CO_2 sample collected five minutes after D-xylose-1-C^{14} was injected was already appreciably labeled. C^{14} was present in expired CO_2 in detectable amounts for six hours and the total $C^{14}O_2$ expired represented 13.5 per cent of the administered label. The similarity of the CO_2 labeling pattern after C^{14} D-xylose and C^{14} D-glucose suggests that these sugars probably share common intermediary pools which have a rapid turnover. The fate of the residual 30 per cent of the administered label, as well as of the lower four carbons of the xylose whose first carbon appeared as CO_2, remains obscure.

From *Nutrition Reviews,* Vol. 16, No. 7, p. 202, July 1958.

Heparin-induced Lipemia Clearing Factor in Rats

Role of the Pancreas in its Production

H. C. Meng, M.D., Ph.D., and June L. Goldfarb, M.D., Nashville

It has long been known that hyperlipemia occurs in many patients with diabetes mellitus.[1-4] The increase in serum lipids is predominantly in triglycerides although cholesterol and phospholipids are also elevated.[5] This condition is aggravated by the presence of acidosis,[6-9] but can be restored to normal by insulin treatment.[7] Recently, an elevation in concentrations of serum lipoproteins has also been found to occur in diabetics,[10-13] but their responses to insulin have been variable.

Warren and LeCompte state that "there can be no question that diabetics are prone to develop atherosclerosis at an earlier age and to a greater extent than other persons."[14] Autopsy findings of various investigators show a much higher incidence of coronary artery disease in diabetics than that in nondiabetics,[15-20] although Bell[20] has found that development of severe vascular lesions is independent of the severity of the diabetes. Thus, a relationship between the disturbance of lipid metabolism and high incidence of atherosclerosis may well exist, although the understanding of the former is far from complete and the etiology of the latter remains obscure.

It is well documented that intravenous injection of heparin is capable of clearing alimentary lipemia and intravenously injected lipoproteins, fatty chyle and synthetic fat emulsions in vivo[21-27] and that plasma obtained after heparin injection hydrolyzes the synthetic triglyceride emulsion or triglyceride component of lipemic plasma in vitro.[28,29] Recently, we have found that the clearing activity of postheparin plasma of human diabetics is decreased.[30] It was postulated that the failure of postheparin plasma of diabetic subjects to clear or hydrolyze triglyceride may, in part, be responsible for the disturbance of lipid metabolism which contributes to the development of atherosclerosis.

From the Department of Physiology, Vanderbilt University, School of Medicine, Nashville, Tennessee. Dr. Goldfarb's present address: Institute for Psychosomatic and Psychiatric Research and Training, Michael Reese Hospital, Chicago, Illinois.

A preliminary report of this work was presented at the Annual Meetings of the American Society for the Study of Arteriosclerosis in Chicago, November 1956.

The present study was undertaken to investigate the postheparin plasma clearing activity of alloxan-diabetic rats and the role of the pancreas in the production of the heparin-induced lipemia clearing factor.

MATERIALS AND METHODS

Young adult male rats of Sprague-Dawley strain weighing 200-250 gm. were used. The animals were under light ether anesthesia during heparin injection, blood withdrawal, pancreatectomy, and alloxanization. Alloxan, 50 mg. per kilogram, was given intracardially or intravenously through a tail vein. An effort at total removal was made in the rats subjected to pancreatectomy. All rats were fed ground Purina chow. In some instances 5 per cent lyophilized beef pancreas or 0.5 per cent Lipormone* was added to the diet as indicated below. Rats with alloxan diabetes were given insulin subcutaneously in long-term experiments. Four to 8 units of insulin were given daily depending on their blood glucose level, and the time of injection was kept regular each day. Both Protamine Zinc insulin and Iletin (Insulin, Lilly) were used and showed no significant differences in their influence upon the clearing activity of postheparin plasma.

Unless otherwise specified, the dosage of heparin was 0.05 mg. per kilogram administered intracardially. All blood samples were withdrawn three to five minutes after heparin and blood was transferred into centrifuge tubes containing sodium citrate kept in ice water. plasma was determined by the method of Meng and gation for twelve minutes at 2,500 r.p.m. in a cold room. The plasmas were used immediately or stored in cold (4° C.). No plasma was stored for more than two or three days.

The in vitro clearing activity of pre- or postheparin plasma was determined by the method of Meng and associates[31] except that 0.5 ml. plasma was used in the present work. The decrease in optical density was recorded at intervals during a two-hour incubation period

*Lipormone, purified lipocaic of pancreas, was kindly supplied by Laboratoire Choay, Paris, France.

at 37° C. In some experiments the unesterified fatty acids were also determined in addition to the decrease in optical density. The method of Borgstrom[32] was used for unesterified fatty acid determination.

Blood glucose was measured forty-eight hours after alloxanization and whenever blood was withdrawn for plasma clearing activity. Nelson's colorimetric method modified by Somogyi[33] was used for determining blood glucose. The animals were not fasted prior to blood withdrawal for blood glucose determination.

RESULTS

Clearing and lipolytic activities of pre- and postheparin plasma of normal rats. It is shown in figure 1 that plasma obtained after intravenous injection of heparin (postheparin plasma) into normal rats was capable of clearing or decreasing the turbidity of synthetic triglyceride emulsion in vitro. This clearing was accompanied by an increase in the production of unesterified fatty acids. Both clearing and lipolytic activities of preheparin plasma were nil as judged by the slight decrease in optical density and the production of unesterified fatty acids. The average blood glucose of these two groups of rats (seven received no heparin and fourteen received heparin) was 113 mg. per cent, ranging from 68 to 155.

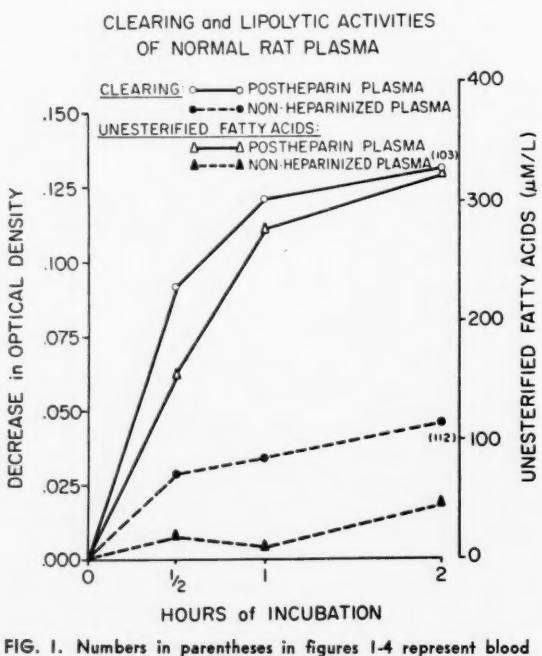


FIG. 1. Numbers in parentheses in figures 1-4 represent blood glucose in milligrams per cent.

Clearing and lipolytic activities of pre- and postheparin plasma of alloxan-diabetic rats. The clearing activity of the preheparin plasma of alloxan-diabetic rats was similar to that of normal rats receiving no heparin. However, the clearing activity of the postheparin plasma was markedly decreased. In correlation with the poor clearing activity, there was no increase in the production of unesterified fatty acids (figure 2). Increasing the heparin dosage from 0.05 mg. to 2.0 mg. per kilogram slightly increased the clearing activity

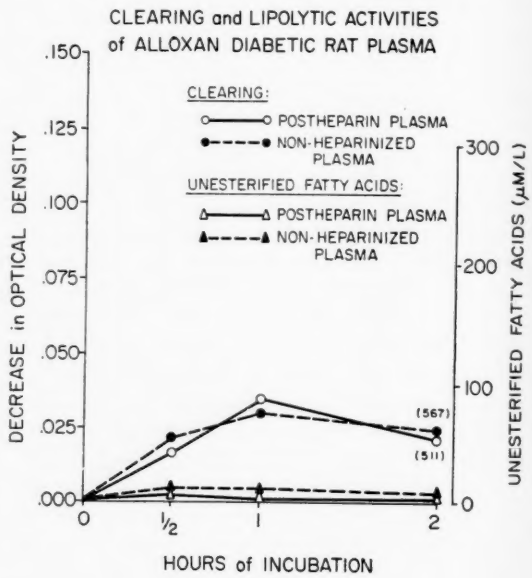


FIGURE 2

of postheparin plasma of alloxan-diabetic rats (figure 3). The over-all average of blood glucose of these two groups of rats (ten received heparin and ten no heparin) was 556 mg. per cent with the range of 252-830.

Clearing activity of postheparin plasma of depancreatized rats. The depancreatized rats were divided into two groups. (1) Nondiabetic with blood glucose of 140 mg. per cent or less, thirteen rats (blood sugar ranged from seventy-six to 140 with an average of 107); and (2) diabetic with blood glucose more than 140 mg. per cent (twelve rats). The diabetic group was subdivided into two groups: (a) six rats received 0.05 mg. heparin per kilogram, and (b) six received 2.0 mg. heparin per kilogram. The blood glucose level ranged from 152 to 405 mg. per cent with an average of 276. Blood glucose and postheparin plasma clearing were determined between one to four weeks after pancreatectomy. The results are shown in figure 4. It can

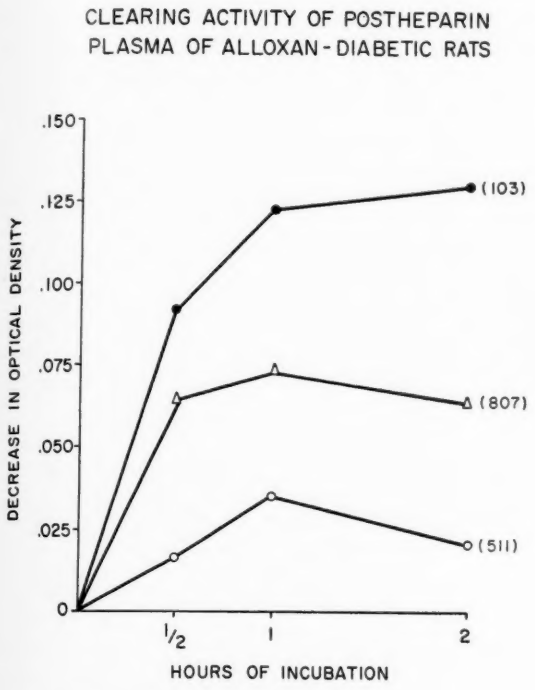


FIG. 3. [103]Normal rats 0.05 mg. heparin/kg.
[807]Alloxan diabetic rats 2 mg. heparin/kg.
[511]Alloxan diabetic rats 0.05 mg. heparin/kg.

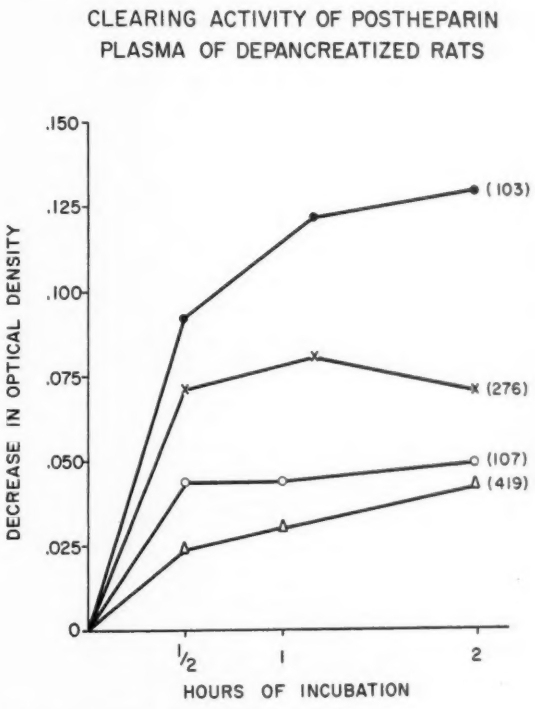


FIG. 4. [103]Normal rats 0.05 mg. heparin/kg.
[276]Depancreatized diabetic rats 2 mg. heparin/kg.
[107]Depancreatized nondiabetic rats 0.05 mg. heparin/kg.
[419]Depancreatized diabetic rats 0.05 mg. heparin/kg.

be seen that the clearing activity of postheparin plasma of depancreatized rats was also significantly reduced even in the nondiabetic group. The clearing activity of postheparin plasma of depancreatized diabetic rats receiving 0.05 mg. heparin per kilogram was further reduced. As in the alloxan-diabetic rats, an increase in the heparin dosage to 2.0 mg. per kilogram improved but did not completely correct the impairment in clearing.

Effect of insulin on the clearing activity of postheparin plasma of alloxan-diabetic rats. A total of twelve rats was included in this experiment. The blood glucose of these rats forty-eight hours after alloxanization averaged 436 mg. per cent ranging from 156 to 830. These rats were then given 4 to 8 units of insulin subcutaneously daily for two to six weeks after which blood glucose and clearing activity of postheparin plasma were determined. The blood glucose ranged from 53 to 146 mg. per cent with an average of 92 mg. The clearing activity of postheparin plasma was improved over that of alloxan-diabetic rats not being controlled with insulin, but remained significantly lower than that of normal rats (figure 5).

Effects of insulin plus lyophilized beef pancreas feeding on the clearing activity of postheparin plasma of alloxan-diabetic rats. Eighteen diabetic rats were maintained on 4 to 8 units of insulin daily beginning forty-eight hours after alloxanization. The amounts of insulin given depended on the level of blood glucose. The animals were immediately fed ground Purina chow containing 5 per cent lyophilized beef pancreas. Eleven rats were on this regimen for four to six weeks and seven for eleven to sixteen days. The blood glucose determined forty-eight hours after alloxanization averaged 537 mg. per cent (ranging 163-906) and 458 mg. per cent (ranging 373-540) for the former (long-term) and the latter (short-term) groups respectively. At the end of the appropriate period, blood glucose was again measured and clearing activity of postheparin plasma determined. It was found that the blood glucose of the long-term group averaged 164 mg. per cent (ranging 47-397) and that of the short-term group averaged 44 mg. per cent (ranging 28-68). The clearing activity

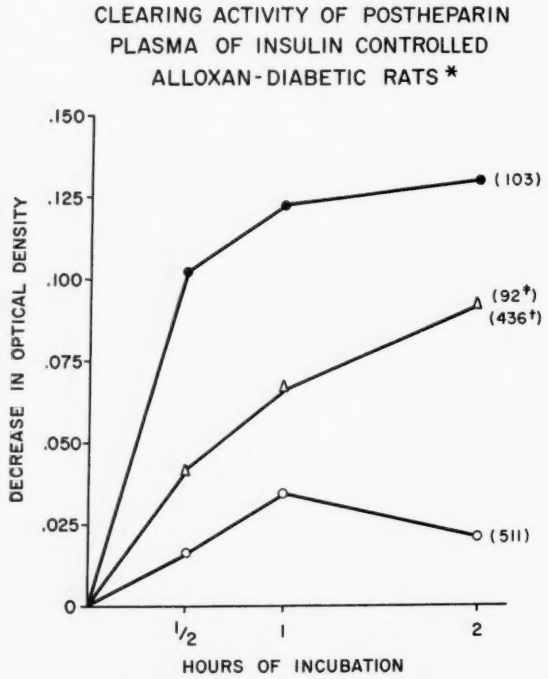


FIG. 5. *All rats were given 0.05 mg. heparin/kg.
†Blood glucose forty-eight hours after alloxanization.
‡Blood glucose at the time of blood withdrawal for clearing activity after two to eight weeks of insulin treatment.
[103]Normal rats.
[92, 436]Insulin controlled alloxan-diabetic rats.
[511]Alloxan-diabetic rats.

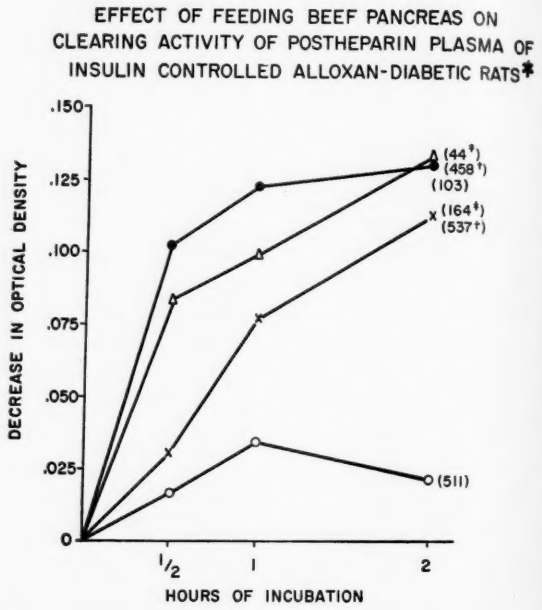


FIG. 6. Insulin controlled diabetic rats were fed beef pancreas for four to six weeks. Insulin controlled alloxan-diabetic rats were fed beef pancreas eleven to sixteen days.
*All rats were given 0.05 mg. heparin/kg.
†Blood glucose forty-eight hours after alloxanization.
‡Blood glucose at the time of blood withdrawal for clearing activity determination.
[44]Insulin controlled diabetic rats.
[103]Normal rats.
[164, 537]Insulin controlled alloxan-diabetic rats.
[511]Alloxan-diabetic rats.

of postheparin plasma was corrected from the marked low level of diabetic rats to that of normal animals. Feeding a diet containing 5 per cent lyophilized beef pancreas for eleven to sixteen days was almost as effective as for four to six weeks (figure 6).

Effect of insulin plus feeding of Lipormone on the clearing activity of postheparin plasma of alloxan-diabetic rats. The blood glucose of nine rats determined forty-eight hours after injection of alloxan averaged 528 mg. per cent (ranging 242-810). The animals were then given 4 to 8 units of insulin daily and fed Purina chow containing 0.5 per cent Lipormone. After they were maintained on this regimen for three to five weeks, blood glucose and clearing activity of postheparin plasma were determined. It was found that the clearing activity of postheparin plasma was also markedly increased over that of the diabetic rats as in those receiving pancreas diet. The blood glucose ranged from 75-363 mg. per cent with an average of 208 at the time of blood withdrawal for clearing determinations.

Summary of clearing activity of postheparin plasma of various groups of rats. Figure 7 summarizes the clearing activity of postheparin plasma of various experimental groups of rats. The results represent the mean decrease in optical density (clearing activity) of the buffer system containing pre- or postheparin plasma and synthetic triglyceride emulsion after two-hour incubation at 37° C. A comparison between the clearing of postheparin plasma of normal rats and that of other groups is shown in table 1.

Lack of correlation between blood glucose level and clearing activity of postheparin plasma of alloxan-diabetic rats. In figure 8 a vertical line is drawn at the blood glucose level of 140 mg. per cent and a horizontal line at the decrease in optical density of 0.10. It can be seen that the clearing activity of postheparin plasma did not correlate with the blood glucose levels in rats of various groups. It is of interest to note that all normal

TABLE 1

Clearing activity of postheparin plasma of various groups of rats: a comparison between normal and other groups

Experimental group	Decrease in optical density (in two hours) Mean ± S.E.	Experimental group	Mean ± S.E.	Difference in mean	P
		Alloxan-diabetic (10)	0.021 ± 0.008*	0.109	0.001
		Alloxan-diabetic (10)	0.064 ± 0.018†	0.066	0.001
		Depancreatized nondiabetic (13)	0.051 ± 0.009*	0.079	0.001
Normal (14)	0.130 ± 0.008	Depancreatized diabetic (6)	0.043 ± 0.016*	0.087	0.001
		Depancreatized diabetic (6)	0.070 ± 0.022†	0.060	0.01
		Alloxan-diabetic + insulin (12)	0.095 ± 0.012*	0.035	0.02
		Alloxan-diabetic + insulin and pancreas diet‡ (11)	0.134 ± 0.017*	0.004	0.8
		Alloxan-diabetic + insulin and pancreas diet§ (7)	0.113 ± 0.005*	0.017	0.05
		Alloxan-diabetic + insulin and Lipormone diet (9)	0.112 ± 0.007*	0.018	0.1

*0.05 mg. heparin per kg. †2.0 mg. heparin per kg. ‡On diet containing pancreas for four to six weeks.
§On diet containing pancreas for eleven to sixteen days.
Numbers in parentheses in columns 1 and 3 (Experimental Group) represent the number of animals.

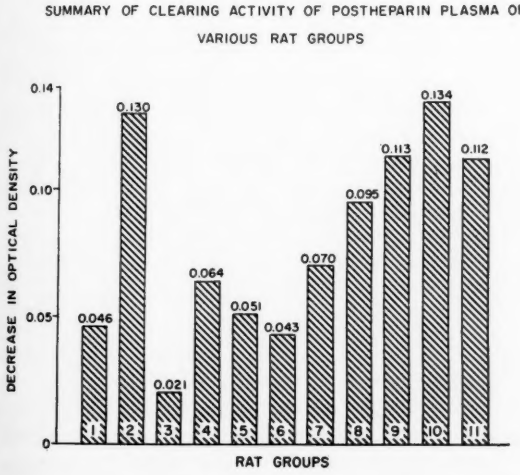


FIG. 7. Figures above the bars are mean decrease in optical density after two-hour incubation at 37° C. of various groups of rats. 1. Nonheparinized plasma of normal rats 2. Postheparin plasma of normal rats 3. Postheparin plasma of alloxan diabetic rats receiving 0.05 mg. heparin/kilogram 4. Postheparin plasma of alloxan-diabetic rats receiving 2.0 mg. heparin/kilogram 5. Depancreatized nondiabetic rats receiving 0.05 mg. heparin/kilogram 6. Depancreatized diabetic rats receiving 0.05 mg. heparin/kilogram 7. Depancreatized diabetic rats receiving 2.0 mg. heparin/kilogram 8. Insulin controlled alloxan-diabetic rats 9. Insulin controlled alloxan-diabetic rats fed beef pancreas for four to six weeks 10. Insulin controlled alloxan-diabetic rats fed beef pancreas for eleven to sixteen days 11. Insulin controlled alloxan-diabetic rats fed Lipormone. All rats in groups 8, 9, 10 and 11 were given 0.05 mg. heparin/ kilogram.

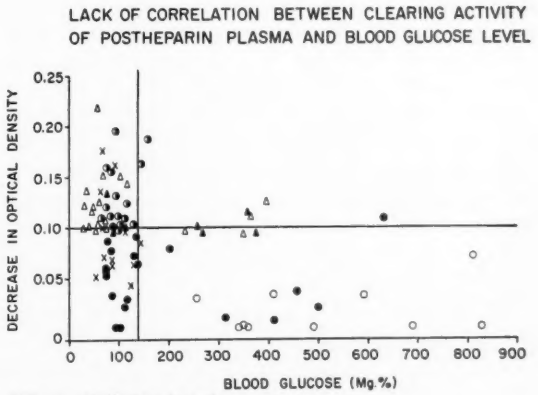


FIG. 8. Half-closed circle—normal rats.
Open circle—alloxan-diabetic rats.
Closed circle—depancreatized rats.
×—insulin controlled alloxan-diabetic rats.
Open triangle—insulin controlled alloxan-diabetic rats plus beef pancreas feeding.
Closed triangle—insulin controlled alloxan-diabetic rats plus Lipormone feeding.

rats are in or near the upper left quadrant, and all alloxan-diabetic rats are in the lower right quadrant. However, the clearing activity of postheparin plasma of the depancreatized rats was all below the horizontal line except in two instances, yet their blood glucose levels ranged from lower normal to as high as 630 mg. per cent. The blood glucose of the insulin controlled alloxan-diabetic rats ranged from 55 to 145 mg. per cent; but the clearing activity of postheparin

plasma was below the horizontal line in eight of the twelve animals. It was further noted that the clearing activity of postheparin plasma of insulin controlled alloxan-diabetic rats fed a diet containing lyophilized beef pancreas or Lipormone was all above or near the horizontal line, yet the blood glucose of these rats ranged from 28-400 mg. per cent.

DISCUSSION

The finding that failure of postheparin plasma of alloxan diabetic rats to clear or lipolyze the synthetic triglyceride emulsion in vitro is in accord with our observations obtained in patients with diabetes mellitus.[20] Since the postheparin plasma of depancreatized rats was also incapable of exerting the clearing activity, a relation between pancreas and the clearing phenomenon seems apparent. It is not known, however, whether alloxanization or pancreatectomy causes a decrease in the production of the heparin-induced lipemia clearing factor per se or whether these procedures provoke a reaction which hinders the production of clearing factor or inhibits its activity. Increasing dosage of heparin does improve the clearing activity of postheparin plasma of both alloxanized and depancreatized rats. These findings would suggest that the production of the heparin-induced clearing factor can be enhanced either by stimulating the remaining normal cells of the pancreas or by affecting cells of other organs. We have shown that addition of heparin to the perfusate of the isolated normal rat heart is capable of producing clearing factor.[24] It is not known if heparin could induce the production of clearing factor in a perfused diabetic rat heart.

The fact that insulin treatment improved the clearing activity of postheparin plasma of alloxan-diabetic rats would indicate that the defect in producing the clearing factor can be partially corrected. This improvement might have been due to the increased utilization of glucose alone or in combination with other metabolic processes. However, insulin was unable to correct completely the impairment in clearing by postheparin plasma although it lowered the blood glucose level to normal. The findings are not surprising since the clearing activity of postheparin plasma did not correlate with the blood glucose level.

It may well be possible that the reduced clearing activity of postheparin plasma in alloxanized or depancreatized rats stems from lack of a certain essential factor ordinarily supplied by the pancreas. Success in correcting this defect of postheparin plasma clearing by means of feeding the animals a diet containing lyophilized beef pancreas constitutes good evidence for such a possibility. The nature of this pancreatic factor is not known. The defect in clearing in alloxan-diabetic rats could also be corrected by feeding of Lipormone, a purified preparation of lipocaic of Dragstedt.[25] At present it is not possible to state whether the two substances are identical. However, since it has been observed in this laboratory that destruction of acinous tissues of the pancreas did not reduce the clearing activity of postheparin plasma,[26] the islets seem to be the source of this factor.

The findings reported must be considered preliminary in nature. Further studies are necessary to investigate the role of pancreas, particularly the islets of Langerhans in the production of heparin-induced lipemia clearing factor in vivo and in lipid metabolism.

SUMMARY

The clearing activity of postheparin plasma of alloxan-diabetic and depancreatized rats was markedly reduced. Increase in heparin dosage administered to, or insulin treatment of, these rats improved but failed to correct this defect. Insulin treatment plus feeding of a diet containing lyophilized beef pancreas or Lipormone, a purified lipocaic, was capable of restoring the postheparin plasma clearing of alloxanized rats to that of normal animals. It is suggested that a pancreatic factor may be necessary in the in vivo production of the heparin-induced clearing factor.

SUMMARIO IN INTERLINGUA

Le Rolo Del Pancreas In Le Production, Inducite Per Heparina, De Factor De Clarage De Lipemia

Le activitate de clarage in plasma post-heparinic ab rattos alloxano-diabetic e dispancreatisate esseva marcatemente reducite. Augmento del dosage de heparina o administration de insulina a iste rattos meliorava sed non normalisava le activitate in question. Le administration de insulina in association con un dieta continente lyophilisate pancreas bovin o liphormon (un purificate lipocaico) esseva capace a restaurar le activitate de clarage in plasma post-heparinic ab alloxanisate rattos al nivello trovate in animales normal. Es suggerite que un factor pancreatic es possibilemente necessari in le production in vivo del factor de clarage inducite per heparina.

ACKNOWLEDGMENT

The work reported here was supported by research grants from the American Heart Association; Eli Lilly and Company, Indianapolis; and Mid-Tennessee Heart Association, Nashville, Tennessee.

The authors wish to express their appreciation to Dr. Margaret P. Martin and Mr. Ben Couch, Department of Preventive Medicine, Vanderbilt University School of Medicine, for statistical analysis of the data presented in table 1.

REFERENCES

[1] Joslin, E. P., Bloor, W. R., and Gray, H.: The blood lipids in diabetes. J.A.M.A. *69*:375-78, 1917.

[2] Rabinowitch, I. M.: The cholesterol content of blood plasma in diabetes mellitus: A statistical study based on two thousand observations in three hundred and eighty-five cases. Arch. Int. Med. *43*:363-71, 1929.

[3] Blix, G.: Studies on diabetic lipemia. I. Acta Med. Scand. *64*:142-74, 1926.

[4] Gray, H.: Lipoids in 1,000 diabetic bloods, with special regard to prognosis. Am. J. Med. Sc. *168*:35-46, 1924.

[5] Pomeranze, J., and Kunkel, H. G.: Serum lipids in diabetes mellitus. Proc. Amer. Diabetes Assoc. *10*:217-31, 1950.

[6] Man, E. B., and Albrink, M. J.: Serum lipids in different phases of carbohydrate metabolism. Yale J. Biol. & Med. *29*: 316-34, 1956.

[7] Hirch, E. F., Phibbs, B. P., and Carbonaro, L.: Parallel relation of hyperglycemia and hyperlipemia (esterified fatty acids) in diabetes. A.M.A. Arch. Int. Med. *91*:106-17, 1953.

[8] Harris, L. V. D., Albrink, M. J., Van Euk, W. F., Man, E. B., and Peters, J. P.: Serum lipids in diabetic acidosis. Metabolism *2*:120-32, 1953.

[9] Herbert, F. K.: Observations on the blood fats in diabetic lipemia. Biochem. J. *29*:1887-93, 1935.

[10] Barach, J. H., and Lowy, A. D.: Lipoprotein molecules, cholesterol and atherosclerosis in diabetes mellitus. Diabetes *1*:441-46, 1952.

[11] Keiding, N. R., Mann, G. V., Root, H. F., Lawry, E. Y., and Marble, A.: Serum lipoproteins and cholesterol levels in normal subjects and in young patients with diabetes in relation to vascular complications. Diabetes *1*:434-40, 1952.

[12] Tuller, E. F., Mann, G. V., Schertenleib, F., Roehring, C. B., and Root, H. F.: The effects of diabetic acidosis and coma upon the serum lipoproteins and cholesterol. Diabetes *3*: 279-86, 1954.

[13] Kolb, F. O., deLalla, O. F., and Goffman, J. W.: The hyperlipemias in disorders of carbohydrate metabolism: Serial lipoprotein studies in diabetic acidosis with xanthomatosis and glycogen storage disease. Metabolism *4*:310-17, 1955.

[14] Warren, S., and LeCompte, P. M.: The Pathology of Diabetes Mellitus. Philadelphia, Lea and Febiger, 1952, p. 146.

[15] Root, H. F., Bland, E. F., Gordon, W. H., and White, P. D.: Coronary atherosclerosis in diabetes mellitus; postmortem study. J.A.M.A. *113*:27-30, 1939.

[16] Stearn, S., Schlesinger, M. J., and Rudy, A.: Incidence and clinical significance of coronary artery disease in diabetes mellitus. Arch. Int. Med. *80*:463-74, 1947.

[17] Clawson, B. J., and Bell, E. T.: Incidence of fatal coronary disease in nondiabetic and diabetic persons. Arch. Path. *48*: 105-06, 1949.

[18] Millard, E. B., and Root, H. F.: Degenerative vascular lesions and diabetes mellitus. Am. J. Digest. Dis. *15*:41-51, 1948.

[19] Liebow, I. M., and Hellerstein, H. K.: Cardiac complications of diabetes mellitus. Am. J. Med. 7:660-70, 1949.

[20] Bell, E. T.: A post-mortem study of 1,214 diabetic subjects with special reference to the vascular lesions. Proc. Am. Diabetes Assoc. *10*:62-82, 1950.

[21] Hahn, P. F.: Abolishment of alimentary lipemia following injection of heparin. Science *98*:19-20, 1943.

[22] Anderson, N. G., and Fawcett, B.: An antichylomicronemic substance produced by heparin injection. Proc. Soc. Exper. Biol. & Med. *74*:768-71, 1950.

[23] Bragdon, J. H., and Havel, R. J.: In vivo effect of antiheparin agents on serum lipids and lipoproteins. Am. J. Physiol. *177*:128-33, 1954.

[24] French, J. E., Morris, B., and Robinson, D. S.: Observations on the removal of chylomicra from the blood. Third Internat. Conference on Biochem. Problems of Lipids. Brussels, 1956, pp. 323-31.

[25] Grossman, M. I., and Strub, I. H.: Effect of heparin on fate of intravenously administered fat emulsion in rats. Proc. Soc. Exper. Biol. & Med. *85*:356-59, 1954.

[26] Meng, H. C., and Youmans, J. B.: Effect of heparin on serum lipids following intravenous administration of fat emulsion in dogs. Proc. Soc. Exper. Biol. & Med. *97*:691-93, 1958.

[27] Shoulders, H. H., Jr., Meng, H. C., and Tuggle, S.: Effects of heparin on body temperature and plasma lipids following intravenous administration of fat emulsion in man. J. Lab. and Clin. Med. In press.

[28] Anfinsen, C. B.: On the role of lipemia clearing factor in lipid transport. A Symposium on Fat Metabolism, edited by Victor A. Najjar. Baltimore, the Johns Hopkins Press, 1954, pp. 93-110.

[29] Hollett, C., and Meng, H. C.: Comparison of fat emulsion clearing activities of postheparin plasma and pancreatic lipase. Am. J. Physiol. *184*:428-32, 1956.

[30] Meng, H. C.: Unpublished results.

[31] Meng, H. C., Hollett, C., and Cole, W. E.: Some factors affecting the clearing of neutral fat emulsion by postheparin plasma. Am. J. Physiol. *179*:314-18, 1954.

[32] Borgstrom, B.: On the action of pancreatic lipase on triglycerides in vivo and in vitro. Acta Physiol. Scand. *25*:328-47, 1952.

[33] Somogyi, M.: Notes on sugar determination. J. Biol. Chem. *195*:19-23, 1952.

[34] Meng, H. C.: Production of heparin-induced lipemia clearing factor in perfused rat heart. Third World Congress of Cardiology, Abstract. September, 1958.

[35] Dragstedt, L. R., Prohoska, J. V., and Harms, H. P.: Observations on a substance in pancreas (a fat-metabolizing hormone) which permits survival and prevents liver changes in depancreatized dogs. Am. J. Physiol. *117*:175-81, 1936.

[36] Meng, H. C., and Hadley, J. L.: Further studies of pancreas and the production of heparin-induced lipemia clearing factor. To be published.

Glucose Uptake and Response to Insulin of the Isolated Rat Diaphragm

The Effect of Denervation

Maria Gordon Buse, M.D., and John Buse, M.D.,
with the technical assistance of Eleanor Porter, B.S., Charleston, South Carolina

It has been known for many years that exercise decreases the insulin requirements of diabetics and increases glucose uptake from the blood even in untreated diabetic subjects.[1] Ingle[2,3] demonstrated that severe work can cause a drop in blood sugar to extremely low levels in partially depancreatized diabetic rats receiving no insulin and that the effects of insulin and work are additive. Levine and his co-workers[4] investigated the effect of muscular work on the volume of distribution of non-metabolizable monosaccharides in eviscerated, nephrectomized dogs and showed that exercise promotes the cellular entry of some sugars as does insulin. The sugars which respond to exercise in this preparation are those that also respond to insulin. Huycke and Kruhoffer[5] studied the perfused hindlimb preparation of the cat and observed that muscular exercise increases the rate of disappearance of D-glucose and D-galactose from the extracellular space. Helmreich and Cori[6] observed that tetanic contraction of skeletal muscle causes accumulation of free glucose within the muscle cells of intact animals. These authors[7] also compared the rate of penetration of infused pentoses into resting and exercising gastrocnemius muscles of nephrectomized rats. They found that pentoses penetrate the intracellular space of working muscles at an accelerated rate and that the effect of exercise parallels that of insulin. These results were recently confirmed in the cat by Sachs and his colleagues[8] who also noted the additive effect of insulin and exercise.

These data demonstrate that exercise facilitates the entry of sugars into muscle cells. The mechanism of this action is essentially unknown. Levine and his co-workers[4] postulated that exercising muscle produces a humoral agent which accelerates the transfer of certain sugars across cell surface barriers. The work of Cori and others[5–9] suggests that the changes in permeability affect primarily the working muscles per se.

Park and Johnson[9] found rhythmically contracting muscles of the rat (heart, diaphragm) to be more responsive to insulin than the gastrocnemius, as measured by the accumulation of free glucose in muscle after infusion of glucose and insulin.

The isolated rat diaphragm is extensively used in bio-assays of insulin activity. As the diaphragm is a constantly working muscle we investigated the effect of antecedent paralysis in vivo on the subsequent glucose uptake in vitro and response to insulin of this tissue. We used diaphragms subjected to short-term denervation (twelve hours to eight days) in order to avoid using muscles with gross atrophy.

MATERIAL AND METHOD

Male Wistar rats between 75 and 150 gm. body weight were used. In individual experiments the body weights of rats were as similar as possible, generally within 10 gm. All animals were kept in the laboratory for at least five days prior to the experiment and maintained on Purina rat pellets and water ad libitum. They were fasted for sixteen hours prior to the experiments, except in one study, which will be indicated in the text.

Operative procedures. Right or left unilateral phrenicotomies were carried out under light ether anesthesia. In early experiments the phrenic nerve was exposed through a thoracotomy incision. A cervical approach was chosen in subsequent studies. Identical results were obtained with both technics. In each operation the nerve was isolated and identified by stimulation with faradic current prior to severing it with scissors. The complete paralysis of one hemidiaphragm was verified visually through a laparotomy incision before excising the diaphragm for study. In sham operated controls the phrenic nerve was exposed through a thoracotomy but not cut.

Presented by title at the Eighteenth Annual Meeting of the American Diabetes Association in San Francisco, June 21-22, 1958.

From the Department of Medicine, Medical College of South Carolina, Charleston, South Carolina.

The animals were in good physical condition after operation and prior to the experiments.

The determination of glucose uptake and response to insulin in vitro. The technic described by Vallance-Owen and Hurlock[13] for measurement of plasma insulin activity was used. Rats were killed by a blow on the head. The hemidiaphragms were excised separately and placed in chilled buffer solution (10 to 15° C.) for ten minutes. The diaphragms were blotted gently and placed in Warburg vessels containing 2 ml. of incubation medium, with or without insulin. The vessels were gassed for five minutes with a mixture of 95 per cent O_2 and 5 per cent CO_2, stoppered, and incubated for ninety minutes at 37° C. in a Warburg apparatus (80 cycles/min.). Incubation medium: Gey and Gey[14] balanced salt solution ($NaHCO_3$ buffer) plus glucose (300 mg. per cent). Insulin:* Five times recrystallized zinc insulin (Lilly), 0.001 U/ml. medium. At the end of the incubation period glucose in the medium was determined by the method of King.[15] The diaphragms were dried in an oven for two hours at 110° C. The glucose uptake was calculated in milligrams/gram diaphragm (dry weight).

Binding of iodine^{131}-labeled insulin by diaphragms in vitro. Hemidiaphragms were incubated in balanced salt solution[14] plus glucose (300 mg. per cent) and I^{131} insulin (Abbott Laboratories). The insulin concentration varied between 0.001 and 0.004 U. of insulin and the specific activity between 0.16 and 0.27 μc/ml. medium.

The hemidiaphragms were placed in tared Warburg vessels containing 2 ml. of medium. The vessels were reweighed prior to incubation to determine the wet weight of the tissue.

After incubation each hemidiaphragm was washed in four changes of 25 to 50 ml. saline for a total period of sixteen minutes, as described by Stadie.[16] This procedure adequately eliminates all radioactive material that is not firmly bound to the tissue.

The hemidiaphragms were blotted and digested individually in 1 ml. 30 per cent KOH solution for thirty minutes at 100° C., neutralized with I N H_2SO_4, and diluted to 10 ml. with distilled water. Two milliliter aliquots of these samples and of the medium were counted in a scintillation wellcounter. The total counts corresponding to each hemidiaphragm were calculated as counts per gram wet weight and divided by the counts contained in 1 ml. medium prior to incubation. Results were expressed as the ratio of concentration of bound insulin to that of the medium.

Glycogen determinations. Under light ether anesthesia rats were exsanguinated through the aorta, the diaphragm frozen in situ,[1]* excised, and placed in iced saline.

The hemidiaphragms were trimmed, blotted, and transferred into tared Wassermann tubes containing 1 ml. of 30 per cent KOH, and the wet weight determined by difference. Glycogen was measured by a slight modification of the technic of Good and his associates.[17] Values are expressed as milligrams of glucose per gram diaphragm (wet weight).

Presentation of data. Mean values and standard errors are indicated in tables. The number of hemidiaphragms used in determining each mean is shown in brackets.

RESULTS

The effect of paralysis on the glucose uptake in vitro of hemidiaphragms. Results obtained at different time intervals after unilateral phrenicotomy are tabulated in table 1. "Basal glucose uptake" refers to the amount of glucose (milligrams/gram dry weight) consumed by the hemidiaphragm during ninety minutes incubation in the absence of insulin. "Glucose uptake with insulin" refers to the glucose uptake in the presence of 0.001 U. insulin per milliliter medium. "Insulin effect" is the increment in glucose uptake in the presence of insulin, obtained by subtracting the mean basal glucose uptake of each group from the corresponding mean glucose uptake with insulin.

Each value in table 1 represents pooled results of three or more experiments. In each experiment the operated animals were divided into two groups. The hemidiaphragms of one group served for the measurement of basal glucose uptake, and the other group was used for glucose uptake with insulin. One hemidiaphragm of each rat served as control of its paralyzed or sham-operated counterpart as each pair was incubated with or without insulin.

The data indicate clearly that, when incubated with insulin, the glucose uptake of nonparalyzed hemidiaphragms increased approximately 70 per cent over the basal glucose uptake. Paralyzed hemidiaphragms responded to the same amount of insulin with a much smaller increase in glucose uptake. Twelve to twenty hours after unilateral phrenicotomy there was a statistically significant difference ($p<0.01$) between the basal glucose uptake and glucose uptake with insulin of paralyzed hemidiaphragms. However, the insulin effect was less than half of that observed in nonparalyzed hemi-

*Insulin was obtained through the courtesy of Dr. F. B. Peck, Sr., from Lilly Research Laboratories, Indianapolis, Indiana.

*Fluro-Ethyl Spray, Gebauer Chemical Company.

TABLE 1

Effect of denervation on the glucose uptake in vitro of rat hemidiaphragms, with and without added insulin

Group	Time after operation	Basal glucose uptake		Glucose uptake with insulin		Insulin effect	
		Nonparalyzed	Paralyzed	Nonparalyzed	Paralyzed	Nonparalyzed	Paralyzed
Sham operated controls	16 hours to 5 days	39.4 ±2.2 (16)	40.0* ±2.4 (16)	70.6 ±2.2 (20)	70.8* ±2.0 (20)	+31.2	+30.8*
Unilateral phrenicotomy	12-20 hours	42.2 ±2.6 (8)	48.4 ±2.8 (8)	72.8 ±2.0 (10)	58.8 ±1.4 (10)	+30.6	+10.8
	40-72 hours	37.6 ±2.2 (8)	38.8 ±3.0 (8)	63.6 ±3.4 (15)	40.6 ±2.6 (15)	+26.0	+1.8
	5-8 days	48.0 ±6.6 (8)	43.2 ±4.2 (8)	74.4 ±4.0 (10)	41.4 ±2.0 (10)	+26.0	—1.8

Glucose uptakes expressed as glucose milligrams/gram hemidiaphragm (dry weight).
Basal=no insulin added to medium. Insulin=0.001 U/ml. medium. *=sham operated, not paralyzed.

diaphragms. Forty hours to eight days after phrenicotomy there was no statistically significant difference between the mean basal glucose uptake and glucose uptake with insulin of paralyzed hemidiaphragms. Without insulin in the medium, paralyzed and nonparalyzed hemidiaphragms consumed the same amount of glucose; with insulin, the glucose uptake of nonparalyzed hemidiaphragms was always significantly higher ($p<0.01$). In sham-operated controls no significant difference between the two hemidiaphragms was observed with or without insulin.

Figure 1 illustrates the quantitative aspect of the decreased response to insulin of paralyzed hemidiaphragms. The ordinates represent increments in glucose uptake above basal in the presence of insulin, while the insulin concentrations are indicated in the abscissae. The dots represent the mean increments in glucose uptake of hemidiaphragms of nonoperated rats, when incubated in the presence of either 100 or 1,000 micro-units of insulin. (These data were obtained while standardizing the insulin assay method. The technic and incubation medium were identical with those used subsequently for unilaterally denervated diaphragms.) Each dot represents thirty hemidiaphragms; the horizontal lines indicate the standard errors of the means. The cross-hatched bar represents the mean response of nonparalyzed hemidiaphragms of operated rats incubated with 1,000 micro-units of insulin and the open bars the response of paralyzed hemidiaphragms to the same amount of insulin.

It can be observed that the sensitivity of the insulin assay method used is sufficient to detect amounts of insulin smaller than 100 micro-units/ml. In the presence of 1,000 micro-units of insulin per milliliter, the increment in glucose uptake of paralyzed hemidiaphragms was significantly less than the response of control hemidiaphragms to 100 micro-units.

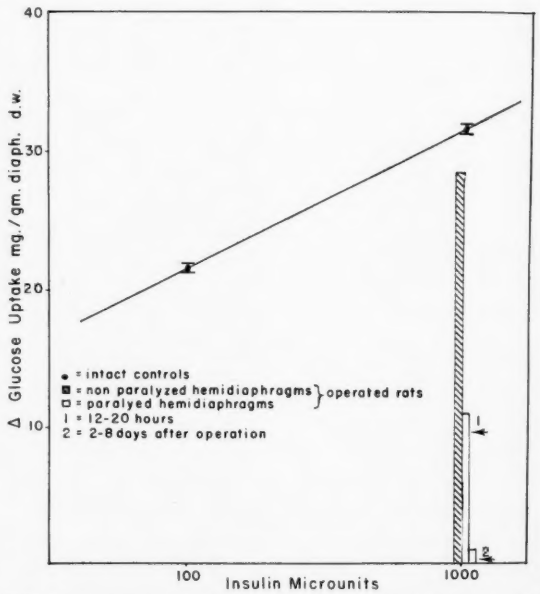


FIG. 1. Increments of glucose uptake above basal by paralyzed and control hemidiaphragms incubated with insulin.

Relation between morphological changes and the decreased response to insulin of denervated hemidiaphragms. The dry weights of paralyzed hemidiaphragms excised at different time intervals after operation were compared with hemidiaphragms of intact controls and

sham-operated rats. Rats between 75 and 134 gm. body weight were included in the study. There was no significant difference between the mean body weights of different groups.

Observing the data listed in table 2 it is evident that no changes in dry weight of paralyzed hemidiaphragms occurred prior to forty-eight hours after operation. On the third day they increased slightly and five to eight days after phrenicotomy the paralyzed hemidiaphragms were significantly heavier ($p<0.01$).

The wet weight of hemidiaphragms was not determined in the above discussed experiments. However, in the course of other studies on paralyzed hemidiaphragms the tissues were weighed immediately after excising them. Comparing the weights of paralyzed and nonparalyzed hemidiaphragms of forty-four rats, between 89 and 139 gm. body weight, three to four days after unilateral phrenicotomy, it was found that the mean wet weight of paralyzed hemidiaphragms was 123 ± 2.6 mg. while the mean weight of their nonparalyzed pairs was 96.7 ± 2.5 mg.

The nitrogen content of diaphragms of ten unilaterally phrenicotomized rats three to five days after operation was also determined and related to the wet weight of the tissues. The mean nitrogen content of paralyzed hemidiaphragms was 2.3 ± 0.1 mg. per cent and that of their nonparalyzed counterpart was 2.2 ± 0.1 mg. per cent. These findings indicate that the observed increase in dry weight is paralleled by a simultaneous increase in wet weight.

Routine histological examination of sections of paralyzed hemidiaphragms, obtained three to five days after operation, did not reveal any striking changes, except the presence of degenerated nerve fibers.

In table 3 data from table 1 are used, but the glucose uptakes are expressed as milligrams of glucose consumed by the hemidiaphragms, without reference to the weight of the tissues. These data are presented to show the absolute glucose uptakes of the hemidiaphragms, irrespectively of eventual changes in their weight. The results obtained are the same as in table 1. There is no difference in basal glucose uptake between paralyzed and nonparalyzed hemidiaphragms. The response to insulin decreases as soon as twelve hours after denervation and is minimal between forty hours and eight days. It is evident that the decreased response to insulin precedes any changes in the weight of paralyzed hemidiaphragms by at least two days, and is independent of the weight of the muscle.

Effect of parenteral insulin on the glucose uptake of paralyzed hemidiaphragms. Unilateral denervation of the diaphragm was carried out in sixteen rats three to four days prior to these studies. Half of the animals underwent left and half right phrenicotomy. On the day of experiment the animals were divided into two groups. The first group was injected intraperitoneally with 0.1 U. of insulin in 0.5 ml. saline, while the second group received saline only. The animals were sacrificed thirty to forty-two minutes after the injection. The two hemidiaphragms were excised separately and handled as described for the preceding experiments. All hemidiaphragms were incubated for ninety minutes in balanced salt solution containing 300 mg. per cent glucose.

Results are summarized in table 4. They demonstrate that the glucose uptake of hemidiaphragms of rats which had not received insulin was not altered by previous denervation. The hemidiaphragms of rats injected with insulin took up more glucose than the controls. However, the response to insulin of paralyzed hemidiaphragms was significantly smaller ($p<0.05$) than that of their nonparalyzed counterparts.

The effect of paralysis on the binding of I^{131}-labeled insulin by rat hemidiaphragms. Stadie and his co-workers[16] demonstrated by using labeled insulin that one of the prerequisites for the action of insulin is its firm binding to muscle tissue.

Experiments were carried out to compare the binding of I^{131}-labeled insulin to paralyzed and nonparalyzed hemidiaphragms in the following situations: (a) during incubation with labeled insulin; (b) after in vivo injection.

In all experiments rats were studied three to four days after unilateral denervation of the diaphragm. Half of the animals underwent right and half left phrenicotomy. The nonparalyzed hemidiaphragm of each rat served as control for its paralyzed counterpart .

A. *In vitro experiments.* The pooled data of five independent experiments are summarized in table 5. Values are expressed as the ratio of concentration of bound insulin to that of the medium. Analysis of the data showed no significant difference in this ratio when the diaphragms were incubated with 0.001, 0.002, or 0.004 units of insulin per milliliter medium. The binding of insulin increased during the first hour of incubation, while there was no significant further increase during the next thirty minutes. At none of the three time intervals studied was a significant difference demonstrable between the I^{131} uptake of paralyzed and nonparalyzed hemidiaphragms.

B. *In vivo experiments*: Under light ether anesthesia I^{131}-labeled insulin diluted to 0.5 ml. with saline was injected into the inferior vena cava of rats (body weight 90 to 100 gm.). The dose of insulin administered was either 0.03 units or

TABLE 2

Effect of denervation on the dry weight of hemidiaphragms

Group	Controls		Paralyzed hemidiaphragms			
	Nonoperated	Sham operated				
Time after operation	—	16 hours to 5 days	10-20 hours	40-48 hours	60-72 hours	5-8 days
Hemidiaphragms mg.	19.7 ±0.2 (36)	20.4 ±1.2 (30)	19.9 ±0.5 (44)	19.4 ±0.6 (26)	21.4 ±0.9 (16)	27.2 ±1.2 (22)

TABLE 3

Effect of denervation on the glucose uptake in vitro of rat hemidiaphragms, with and without added insulin

Time after unilateral phrenicotomy	Basal glucose uptake		Glucose uptake with insulin		Insulin effect	
	Nonparalyzed	Paralyzed	Nonparalyzed	Paralyzed	Nonparalyzed	Paralyzed
12-20 hours	0.90 ±0.06 (8)	0.95 ±0.04 (8)	1.75 ±0.09 (10)	1.29 ±0.05 (10)	+0.85	+0.34
40-72 hours	0.92 ±0.07 (8)	0.91 ±0.04 (8)	1.49 ±0.07 (15)	0.92 ±0.09 (15)	+0.57	+0.01
5-8 days	1.11 ±0.15 (8)	1.12 ±0.12 (8)	1.82 ±0.10 (10)	1.25 ±0.08 (10)	+0.71	+0.13

Glucose uptakes expressed as milligrams of glucose consumed by each hemidiaphragm.
Basal=no insulin added to medium.
Insulin: 0.001 U/ml. medium.

TABLE 4

Effect of parenteral insulin on the subsequent glucose uptake in vitro of paralyzed hemidiaphragms

Group	No insulin		Parenteral insulin 0.1 U/rat	
Hemidiaphragm	Nonparalyzed	Paralyzed	Nonparalyzed	Paralyzed
Glucose uptake	34.0 ±3.0 (8)	37.4 ±3.2 (8)	77.2 ±4.6 (8)	57.8 ±6.2 (8)

Glucose uptake expressed as milligrams/gram (dry weight).

0.25 units per rat, with a specific activity of 2 or 6 μc. The animals were sacrificed thirty minutes after the injection, the hemidiaphragms excised, weighed, washed, and digested as in the earlier in vitro experiments. Aliquots of the digested samples and of the injected insulin solution were diluted and counted in a scintillation wellcounter.

Results (table 6) are expressed as micro-units of insulin bound per gram hemidiaphragm (wet weight). Table 6 demonstrates that there was no decrease in the insulin binding capacity of paralyzed hemidiaphragms. On the contrary, paralyzed hemidiaphragms took up significantly ($p<0.02$) more labeled insulin than their nonparalyzed pairs. This may be due to differences in blood flow between paralyzed and nonparalyzed muscles. Correlating the data on glucose uptake (table 4) and insulin binding (table 6) of denervated hemidiaphragms after the injection of insulin, it is of interest to note that although the paralyzed hemidiaphragms bound approximately 50 per cent more insulin than their nonparalyzed pairs, the insulin effect on glucose uptake was less than half of that observed in nonparalyzed muscles. This emphasizes the decreased response to insulin of paralyzed muscles.

The effect of denervation on the glycogen content of rat hemidiaphragms. It is generally accepted that muscular contractions accelerate glycogenolysis in muscle cells. On the other hand, insulin is known to stimulate

TABLE 5

In vitro binding of I^{131} insulin

Incubation minutes	Ratio of I^{131} insulin bound by hemidiaphragms	
	Nonparalyzed	Paralyzed
30	0.59 ±0.04 (9)	0.67 ±0.04 (9)
60	1.09 ±0.07 (12)	1.25 ±0.11 (12)
90	1.29 ±0.14 (11)	1.25 ±0.07 (11)

I^{131} insulin binding expressed as ratio of counts/gram tissue (wet weight) to counts/milliliter medium.
Insulin concentration: 0.001-0.004 U insulin/milliliter medium in different experiments.

TABLE 6

Binding of parenteral I^{131} insulin

I^{131} insulin microunits injected per rat	I^{131} insulin bound by hemidiaphragms	
	Nonparalyzed	Paralyzed
30	0.16 ±0.02 (9)	0.24 ±0.02 (9)
250	1.37 ±0.05 (9)	1.75 ±0.12 (9)

Bound I^{131} insulin expressed in microunits/gram hemidiaphragm (wet weight).

the synthesis of glycogen in these tissues.

The preceding experiments have shown that denervation diminishes the ability of muscle to respond with an increased glucose uptake to the presence of insulin. Thus the glycogen content of paralyzed hemidiaphragms would be influenced by at least two factors: (a) the lack of exercise decreases glycogenolysis; and (b) the diminished response to insulin should slow down the synthesis of new glycogen.

Glycogen determinations were carried out on hemidiaphragms of nonoperated controls and of operated rats two to four days after right or left phrenicotomy. The animals were either fed ad libitum or fasted for sixteen hours prior to their sacrifice. Table 7 demonstrates the results obtained.

Comparing the glycogen content of hemidiaphragms of fasted, operated rats it is evident that the paralyzed hemidiaphragms contained more glycogen than their nonparalyzed pairs ($p<0.01$). The diaphragms of fasted nonoperated controls contained less glycogen than the paralyzed hemidiaphragms ($p<0.01$) but more ($p<0.01$) than the nonparalyzed hemidiaphragms of operated fasted rats.

Feeding increased the glycogen content of nonparalyzed hemidiaphragms by more than 200 per cent, while the increase observed in paralyzed hemidiaphragms was only 32 per cent of the fasting value. Consequently, in rats fed ad libitum the glycogen content of paralyzed hemidiaphragms was significantly lower ($p<0.01$) than that of their nonparalyzed pairs.

These results can be interpreted in the light of the above mentioned two factors, glycogen breakdown and synthesis. In the fasting state little glucose is available for synthesis of muscle glycogen. As the diaphragm is a rhythmically contracting muscle, glycogen breakdown continues at a high rate. In these circumstances, paralyzed hemidiaphragms contain more glycogen than their working pairs or the diaphragms of nonoperated controls. In rats fed ad libitum the rate of glycogen synthesis and the muscle glycogen content increase. Since this increase is much smaller in paralyzed than in nonparalyzed hemidiaphragms, it is probable that the ability of paralyzed hemidiaphragms to synthesize glycogen is impaired. This correlates well with the decreased response to insulin of these muscles. To establish cause and effect relationship further experiments are necessary.

TABLE 7

The effect of denervation on the glycogen content of hemidiaphragms

Group	Hours fasted	Glycogen content of hemidiaphragms	
		Nonparalyzed	Paralyzed
Controls	16	1.5 ±0.07 (22)	—
Unilateral phrenicotomy	16	1.2 ±0.09 (19)	2.0 ±0.12 (19)
	0	3.7 ±0.17 (24)	2.6 ±0.20 (24)

Glycogen expressed as glucose milligrams/gram (wet weight).

DISCUSSION

There is ample evidence that exercise promotes the entry of glucose and certain nonmetabolizable sugars into muscle cells.[2-9] The work of Hines and his colleagues[10-12] indicates that the absence of exercise also interferes with the carbohydrate metabolism of muscles. These authors showed that (a) the glycogen content of immobilized or denervated rat gastrocnemii decreases; (b) they do not respond with an increase in glycogen to the administration of glucose and insulin; and (c) periodical electrical stimulation prevents the decrease in glycogen content.

The diminished response to insulin of paralyzed hemidiaphragms could be due either to lack of activity or denervation per se. The first hypothesis seems more likely as the decreased insulin sensitivity can be easily demonstrated twelve hours after denervation, when degenerative changes have not yet occurred in muscle. Preliminary experiments conducted in this laboratory indicate that incubation with acetylcholine (5 μg./ml. medium) does not restore the response to insulin of paralyzed hemidiaphragms, but that electrical stimulation in vivo restores their subsequent sensitivity to insulin in vitro. Studies in vivo[7] showed that stimulated muscles maintain their increased permeability to pentoses for thirty minutes after the cessation of muscle work.

This finding is in agreement with the assumption that the changes in glucose uptake in vitro described in our study may be due to the previous state of activity or inactivity of the hemidiaphragms.

No difference in basal glucose uptake was observed in our experiments between paralyzed and nonparalyzed diaphragms, while it has been repeatedly described[2-8] that exercise enhances the permeability of muscle to sugars in the absence of insulin. Comparison is difficult as the experimental conditions of these studies[2-8] differed in many respects from ours. However, the following factors deserve consideration:

1. In each of the mentioned studies[2-8] it was noted that in order to obtain an increase in the permeability to sugars without insulin the muscle has to carry out a considerable amount of work. Gastrocnemii were stimulated (three to five times per second) lifting a weight which represented 10 to 25 per cent of the animals' body weight. The amount of work performed by these preparations must have been far in excess of that carried out by a hemidiaphragm while contracting under physiological conditions. It is possible that the first mechanism by which muscle adapts to the increased caloric requirements of moderate exercise is by increasing its responsiveness to insulin, and only under severe stress, when this mechanism proves insufficient, does the "basal" permeability to glucose increase.

2. Several authors used intact,[5,6,8] alloxan diabetic,[7] or partially depancreatized[2,3] animals in their studies, which presumably had small amounts of circulating insulin. It is possible that the observed effect of exercise on muscle permeability was due to the increased responsiveness of working muscles to endogenous insulin.

3. Since glucose was used as substrate in this study, the results cannot be freely compared with those obtained in experiments with pentoses and D-galactose.[4,6,8] It should be considered that different mechanisms may regulate the permeability of working muscles to glucose and to nonmetabolizable sugars.

This study is in accord with the observations[5-8] that exercise enhances the permeability to sugar of muscles by a local action on the working muscle per se. It is possible, however, that in addition exercising muscles produce a humoral factor, as suggested by Levine.[4]

The difference in the response to insulin between paralyzed and nonparalyzed hemidiaphragms was not due to decreased binding of insulin by denervated muscles as shown in experiments with I^{131}-labeled insulin in vivo and in vitro.

Paralyzed hemidiaphragms were found to have less capacity to increase their glycogen content after feeding, and the glycogen content of denervated hemidiaphragms was lower than that of their working pair in fed animals. These observations agree with studies by Hines and his co-workers[10-12] using denervated rat gastrocnemii and were interpreted to be due to impaired glycogen synthesis. However, these authors also observed that paralyzed gastrocnemii contain less glycogen than controls in fasted animals, which did not occur in the case of the paralyzed hemidiaphragm. This apparent discrepancy can be interpreted by considering that contrary to the gastrocnemius the diaphragm is a continuously working muscle. In control (working) diaphragms of fasted rats the rate of glycogenolysis may exceed the rate of synthesis, resulting in a glycogen content less than that of denervated (inactive) hemidiaphragms.

SUMMARY

The glucose uptake in vitro and responsiveness to insulin of rat diaphragms were studied twelve to 200 hours after unilateral denervation.

Intact and paralyzed hemidiaphragms were incubated in buffer containing 300 mg. per cent glucose with or without added insulin. The amount of glucose consumed during ninety minutes' incubation was related to the tissues' dry weight.

The glucose uptake of paralyzed and control hemidiaphragms was identical in the absence of insulin. When insulin (0.001 U. per milliliter) was added to the medium, the glucose uptake of paralyzed hemidiaphragms increased less than that of controls. Dose response studies indicated that the insulin sensitivity decreased to one tenth of normal twelve hours after denervation. Similarly, after injecting rats in vivo with insulin, paralyzed hemidiaphragms when subsequently assayed in vitro consumed less glucose than controls.

No impairment of binding of insulin labeled with I^{131} was observed in denervated hemidiaphragms.

After feeding, the glycogen content increased 30 per cent in denervated hemidiaphragms and 200 per cent in controls.

The insulin binding capacity of paralyzed hemidiaphragms is unimpaired while their metabolic response to the hormone is decreased, due to inactivity or denervation. The decreased response to insulin preceded detectable morphological changes in muscle. It is suggested that increased insulin responsiveness may represent a mechanism of adaptation by which muscles obtain the necessary sources of energy during exercise.

SUMMARIO IN INTERLINGUA

Le Acceptation De Glucosa E Le Responsa A Insulina Per Isolate Diaphragmas De Rattos

Le acceptation de glucosa in vitro e le responsivitate a insulina per diaphragmas de rattos esseva studiate ab dece-duo usque a duo centos horas post le effectuation de disnervation unilateral. Intacte e paralysate hemidiaphragmas esseva incubate in un solution tampon que contineva 300 mg. pro cento de glucosa con o sin le addition de insulina. Le quantitate de glucosa consumite durante novanta minutas de incubation esseva relationate al peso sic del diaphragma.

Le acceptation de glucosa per paralysate e intacte hemidiaphragmas esseva identic in le absentia de insulina. Quando insulina esseva addite al medio (in un concentration de 0,001 unitates per millilitro), le acceptation de glucosa per paralysate hemidiaphragmas esseva minus marcatemente augmentate que illo observate in le caso del hemidiaphragmas de controlo. Studios del responsa a varie dosages indicava que le sensibilitate a insulina decresceva a un decimo del valor normal intra dece-duo horas post le disnervation. Similemente, post le injection de insulina in rattos in vivo, paralysate hemidiaphragmas, subsequentemente investigate in vitro, consumeva minus glucosa que hemidiaphragmas de controlo.

Nulle reducite ligation de insulina a I^{131} esseva observate in hemidiaphragmas disnervate.

Post alimentation, le contento de glycogeno cresceva per 30 pro cento in hemidiaphragmas disnervate e per 200 pro cento in hemidiaphragmas de controlo.

Le capacitate de ligar insulina remane intacte in hemidiaphragmas paralysate durante que lor responsa metabolic a ille hormon es reducite in consequentia de inactivitate o de disnervation. Le reducite responsa a insulina precedeva detegibile alterationes morphologic in le musculos. Es formulate le opinion que le augmentate responsivitate a insulina representa possibilemente un mechanismo de adaptation per que le musculos acquire le energia necessari in exercitio.

ACKNOWLEDGMENT

Thanks are due to Dr. Ollie Smithwick for assistance in some experiments and to Drs. Melvin H. Kniseley and William M. McCord for their encouragement and support. This study was supported initially by grants-in-aid from the Charleston Scientific and Cultural Fund and the American Medical Association (#247) and mainly by the National Institutes of Health (#A-2001), U.S. Public Health Service.

REFERENCES

[1] Joslin, E. P., Root, H. F., White, P., and Marble, A.: Treatment of Diabetes Mellitus (9th ed.). Philadelphia, Lea and Febiger, 1952.

[2] Ingle, D. J., Nezamis, J. E., and Rice, K. L.: Work output and blood glucose values in normal and diabetic rats subjected to the stimulation of muscle. Endocrinology *46*:505, 1950.

[3] Ingle, D. J., Nezamis, J. E., and Morley, E. H.: Work output and blood glucose values in severely diabetic rats with and without insulin. Am. J. Physiol. *165*:469, 1951.

[4] Goldstein, M. S., Mullick, V., Huddlestun, B., and Levine, R.: Action of muscular work on transfer of sugars across cell barriers: comparison with action of insulin. Am. J. Physiol. *173*:212, 1953.

[5] Huycke, E. J., and Kruhoffer, P.: Uptake of hexoses by muscle cells. Acta Physiol. Scand. *34*:232, 1955.

[6] Helmreich, E., and Cori, C. F.: Some problems of permeability of tissue cells to sugars. Ciba Foundation Colloquia on Endocrinology *9*:227, 1956.

[7] Helmreich, E., and Cori, C. F.: Studies of tissue permeability I. The distribution of pentoses between plasma and muscle. J. Biol. Chem. *224*:663, 1957.

[8] Sacks, J., and Smith, J. F.: Effects of insulin and activity on pentose transport into muscle. Am. J. Physiol. *192*:287, 1958.

[9] Park, C. R., and Johnson, L. H.: Effect of insulin on transport of glucose and galactose into cells of rat muscle and brain. Am. J. Physiol. *182*:17, 1955.

[10] Hines, H. M., and Knowlton, G. C.: Changes in the skeletal muscle of the rat following denervation. Am. J. Physiol. *104*:379, 1933.

[11] Hines, H. M., and Knowlton, G. C.: The role of the nervous system in the regulation of the glycogen metabolism of skeletal muscle. Am. J. Physiol. *111*:243, 1935.

[12] Lazere, B., Thomson, J. D., and Hines, H. M.: Studies on the glycogen metabolism of atrophic and regenerating muscle. Am. J. Physiol. *138*:357, 1942.

[13] Vallance-Owen, J., and Hurlock, B.: Estimation of plasma insulin by the rat diaphragm method. Lancet *1*:68, 1954.

[14] Gey, G. O., and Gey, M. K.: The maintenance of human normal cells and tumor cells in continuous culture. Amer. J. Cancer *27*:45, 1936.

[15] King, E. J.: Microanalysis in Medical Biochemistry. London, 1951.

[16] Stadie, W. C., Haugaard, N., and Vaughan, M.: Studies of insulin binding with isotopically labeled insulin. J. Biol. Chem. *199*:729, 1952.

[17] Good, C. A., Kramer, H., and Somogyi, M.: The determination of glycogen. J. Biol. Chem. *100*:485, 1933.

Special Article

Physiologic Aspects of Hunger and Satiety

Theodore B. Van Itallie, M.D., New York

Lusk[1] once pointed out that early nutritional science received considerable impetus from the common-sense observation that man maintains his weight at a fairly constant level despite ingestion of large quantities of food. This observation impelled nutritionists of the nineteenth century to study foodstuffs, their chemical composition and their role in metabolism. They also investigated the excreta and the "invisible exhalations" of the body. In this way, foundations for modern organic and biological chemistry were laid. However, the mechanism responsible for keeping weight constant was not accessible to systematic study at the time, and, despite considerable curiosity about the intricacies of energy balance regulation, little progress was made in understanding this mechanism until the twentieth century.

HYPOTHALAMIC CENTERS

Within the last two decades it has been demonstrated that there is an area in the hypothalamus specifically concerned with the regulation of food intake. When the medial portions of this area are experimentally damaged, food intake increases out of all proportion to energy needs and the experimental animal becomes grossly obese.[2] When lesions are placed in the lateral area the animal may cease to eat and starves to death unless forcibly fed.[3] Electrostimulation of the lateral area induces hyperphagia.[4]

Some investigators have interpreted these results as indicating that there are at least two discrete "centers" in the hypothalamus having to do with energy balance, one a "feeding center" which is directly concerned with feeding behavior and the other a "regulatory center" which governs the activity of the feeding center.

The knowledge that there exists in the hypothalamus a center concerned with regulation of energy balance has stimulated attempts to elucidate the mechanism whereby energy intake presumably is adapted to energy requirements, and the composition of the body is maintained more or less constant.

Investigation of this problem usually has involved two avenues of approach: (1) observations of feeding behavior in experimental animals (rarely eating behavior in man), and (2) attempts to determine how an organism is able to supply information concerning its nutritional status to a regulatory center in the brain.

From the Department of Medicine, St. Luke's Hospital, and the Institute of Nutrition Sciences, Columbia University, New York, New York.

FEEDING PATTERNS IN ANIMALS

Clearly, studies of regulation of food intake require as their basis reliable information about the feeding patterns of animals, normal and abnormal. Conventionally, observations of feeding behavior in laboratory animals have consisted of measurements of the quantity of food eaten in twenty-four-hour periods and of daily changes in body weight. Results of such studies have been reviewed by Mayer.[5]

A much better understanding of how certain animals regulate their food intake recently has been obtained by means of technics developed by experimental psychologists. One such technic that has been applied in the study of feeding behavior utilizes the principle of operant conditioning.[6] For example, when a sensitive lever is placed in the cage of the animal to be studied it is observed that the animal operates this device with an appreciable frequency, even in the absence of a history of reward in this situation. This initial rate of responding is known as the "free operant level." When lever pressing responses are rewarded with pellets of food, their frequency tends to increase. When satiety is present, lever pressing activity declines to the free operant level; as hunger returns, lever pressing activity again increases. It is possible by electronic devices to obtain a cumulative temporal record of pellets delivered to the animal and also to schedule pellet delivery so that the animal must press the lever a predetermined number of times before one pellet is delivered. By methods of this kind, detailed analysis of feeding behavior is possible and an estimate of hunger drive can be obtained.[7]

Using such technics, Anliker and Mayer[8] have shown that over a twenty-four-hour period, the normal mouse displays cyclical changes in rate of feeding. There is an initial phase of rapid feeding, followed by a second phase in which the rate of feeding decreases. The twenty-four-hour cycling is largely absent in mice made

hyperphagic by means of hypothalmic lesions; such animals eat almost constantly at the same rate.

Teitelbaum and Campbell[9] have demonstrated that, when eating a concentrated liquid diet, rats with hypothalamic lesions that are actively gaining weight consume meals about twice as large as those taken by normal rats. Under these circumstances, the hypothalamic rats do not eat more frequently than the normal rats. However, when eating a solid diet in which the calorie concentration is reduced, hypothalamic-hyperphagic rats increase both the size and frequency of their meals. Teitelbaum[10] has shown, in confirmation of results of Miller, Bailey and Stevenson,[11] that hyperphagic rats paradoxically show a decrease in their drive to obtain food.

Thus, it seems that normal mice and rats have well-defined, cyclical feeding patterns with alternating periods of feeding activity and relative quiescence. In the hypothalamic-hyperphagic mouse and rat this cyclical pattern appears to be lost until the "static phase" of obesity is reached. Then it may reappear. It seems that in the hypothalamic-hyperphagic animal an inhibiting effect of the ventromedial area on the feeding (lateral area) center is impaired and that the satiety mechanism as manifested in deceleration of normal feeding activity and in termination of normal-sized meals no longer operates properly.

HUNGER VERSUS SATIETY

In contrast to the controlled observations that have been made on feeding behavior in certain laboratory animals, relatively little scientifically acceptable information is available about the regulation of energy balance and body composition in man. In a recent valuable study by Durnin,[12] the daily individual intake and expenditure of calories were compared in four groups of people who differed widely in their ways of life and in their total energy expenditure. In each of the groups there was no significant relationship between expenditure of calories and intake of calories on the same day, the intake on the subsequent day and the intake two days after. Thus, marked variations in day-to-day food intake seem to occur in man independently of proximate energy expenditure.

In addition to the quantitative information that has been collected relating to energy balance in man, there is, of course, common-sense awareness of the fact that body weight tends to remain fairly constant for prolonged periods and that hunger and satiety occur in regular cycles, punctuated by meals.

It is of interest to examine the frequency distribution curves for body weight that are obtainable for various population groups. In the United States, where dietary calories are available in abundance, such curves generally show skewness with more people on the "overweight" side of the curve than on the "underweight" side.[13] In terms of the regulations under discussion, the mechanism that acts to keep man from getting below a certain relative weight is more sensitive and reliable than the mechanism that keeps him from getting above a certain relative weight. Consequently, as many have discovered by personal experience, it is easier to put on weight than to lose it.

From a teleologic standpoint, such an arrangement makes good sense. Man's environment was not always so organized that it was possible to obtain food at will. Under these circumstances, it was entirely appropriate and, indeed, necessary to consume food for storage in the body as well as to meet immediate energy requirements.

There is also some experimental evidence to support the notion that the brake on "overeating" is not a particularly strong one. For example, Bernstein and Grossman[14] have reported that intravenous and oral glucose administrations sufficient to raise blood sugar appreciably did not significantly decrease consumption of a test meal by human subjects. Janowitz and Hollander[15] in studies of the effect of intragastric feedings on spontaneous food intake in dogs showed that, although inhibition of food intake under such circumstances was almost completely compensatory, this adjustment required relatively long periods of time (weeks) to be accomplished. Thus, although a regulation inhibiting food intake most certainly exists, it seems to be elastic in nature, particularly in regard to individual meals.

SIGNALS OF SATIETY

How is the body able to supply information concerning its nutritional status to a regulatory center in the brain?

Several theories have been proposed in an attempt to answer this question (table 1). Before considering them, it might be well to recall that there are only three pathways or modalities by which the body can signal its nutritional status to a regulatory center in the brain. As shown in table 1, three of the current theories postulate a chemical signal to the regulatory center, one a physical signal, and one, tentatively, a neurally transmitted signal. Of the various contending formulations, only one, the glucostatic theory, has provided a comprehensive explanation of how a regulatory center might be able to mediate between calorie needs and food intake.[16] Several of the other theories do not actually have names in any official sense but have been given designations arbitrarily for purposes of discussion. Of the five theories

TABLE 1

Theories concerning the regulation of food intake

Modality	Theory	Signal*
Chemical	Glucostatic[21]	Availability of blood glucose
	Lipostatic[18]	Concentration of circulating metabolite (nature unspecified)
	"Amino acid pattern"[17]	Pattern of available amino acids in blood
Physical	"Thermostatic"[32]	Specific dynamic action of ingested food
Neural	"Liponeurostatic"[38]	Impulses from fibers innervating adipose cells

* to a regulatory center in the hypothalamus.

listed, only two, the glucostatic and thermostatic, have clearly defined the signal transmitted to the hypothalamus. Mellinkoff[17] in his discussion of the role of blood amino acids in determining satiety has referred to a "pattern" of amino acids as being important but has not identified the pattern. Kennedy[18] has singled out the fat stores as being important in regulating food intake but has not explained how such stores provide the appropriate information to the brain. Discussions about what might be called a "liponeurostatic" theory have been based on interesting but as yet unconfirmed reports of a direct neural connection between the central nervous system and adipose tissue.[19,20]

GLUCOSTATIC THEORY

Since the glucostatic theory has been well defined, it seems appropriate in this brief discussion to concentrate on this theory and consider its virtues and shortcomings in the light of the other theories and in terms of various phenomena that any theory of food intake presumably must explain.

The glucostatic theory[21] proposes that the available carbohydrate supply of the body exerts a regulatory influence on food intake via hypothalamic "glucoreceptors"; the urge to eat increases as carbohydrate stores diminish. The hypothalamus probably cannot obtain direct information concerning the status of the carbohydrate stores; however, the rate of carbohydrate utilization by the body as a whole tends to be directly proportional to the supply of this nutrient. Thus, measurement of the rate at which blood glucose is utilized (peripheral arteriovenous glucose differences) provides indirect information about available carbohydrate supply.

Since the stores of carbohydrate in the body are strictly limited and are depleted much more rapidly than are stores of fat and protein, the carbohydrate supply would seem a logical meter of the immediate caloric status of the individual. It is difficult to imagine hunger in the presence of an adequate carbohydrate supply or satiety in the presence of carbohydrate depletion. Moreover, carbohydrate of all the nutrients seems to set the metabolic "tone" of the body; if such terms can be used in this context, carbohydrate is an "active" nutrient while fat seems to be "passive."[22]

In recent years, considerable evidence has been obtained that can be interpreted as supporting the glucostatic theory. Unfortunately, only a part of this material can be mentioned in the present brief discussion. For example, a number of studies have disclosed an association in time (1) between the experience of hunger and a diminished rate of peripheral glucose utilization, and (2) between satiety and an increased rate of glucose utilization.[23–25] Several reports have indicated that the hypothalamus may indeed contain glucose-sensitive areas.[26,27] On the other hand, critics of the glucostatic theory have contended that the temporal relationship between available glucose and satiety is fortuitous and that the hunger associated with hypoglycemia is in the nature of an "emergency mechanism."[28]

CRITERIA

Assuming for the moment that there exists a center in the brain for regulation of food intake, what precisely does such a center have to account for? It would appear to have to account for at least the following observations: namely, that (1) we seem to eat to maintain a certain body composition, regardless of variations in caloric expenditure; (2) hunger develops if our meals during waking hours are separated by more than four or five hours; and (3) apparently normal satiety can be experienced eventually on a carbohydrate-free diet or fat-free diet, provided dietary calories are adequate.

To what extent can the glucostatic theory and other formulations explain the fact that we seem to eat in order to maintain a certain body composition? It is evident that of the body's major components* the most variable is fat. The lability of stores of triglyceride has been well documented in many studies, including those of Keys et al.[29] on the metabolic effects of prolonged semistarvation.

If, in the long term, we eat to maintain our fat stores at a more-or-less constant level, can such a phenomenon be explained by means of the glucostatic theory? Possibly—if the relative amount of fat used for energy (and hence the rate at which carbohydrate is used) is somehow dependent upon the amount of fat stored in the body. If such a relationship between fat stores and

* In terms of quantity, carbohydrate must be regarded as a minor component.

regulation of energy balance were demonstrable, support also would be available for the lipostatic hypothesis but, presumably, not for the thermostatic or amino acid theories.

Blood sugar fluctuations and rates of carbohydrate utilization reflect in part the composition of the meal eaten at the time and in part the current status of the body with regard to carbohydrate stores. They are not, as far as is known, influenced appreciably by fat stores, unless gross obesity is present. Actually, the rate at which fat stores release unesterified fatty acids for metabolism elsewhere in the body seems largely dependent upon the amount of carbohydrate (not fat) that is available at the time.[22] Thus, it seems clear that blood glucose utilization rates, although they might well account for some of the satiety experienced after a meal and might also account for hunger, cannot readily explain the gradual adjustments in food intake in dogs fed by gastrostomy, or the strong tendency, after a period of semistarvation, to return to a certain body composition over a period of months.

Of considerable interest are the changes in feeding behavior that occur in hypothalamic-hyperphagic rats as they become increasingly obese. It is well known that experimentally-induced hypothalamic obesity consists of two phases: (1) a dynamic phase that begins immediately after hypothalamic injury and during which the hyperphagic rat eats enormous amounts of food and gains weight rapidly; and (2) a static phase, that occurs about a month after the onset of the dynamic phase, and which is characterized by a leveling of weight and a decrease in food intake. During the static phase the obese hyperphagic rat, in contrast to his behavior in the dynamic phase, becomes hyper-reactive to positive (sugar) and negative (quinine) stimulus qualities of the diet and decreases his meal size and the number of meals he eats per day.[30]

Since the only known physiologic difference between the dynamic and static hyperphagic animals is in their degree of adiposity, it might seem that the fat depots exert an inhibitory influence on food intake under these circumstances. However, the pathways involved remain obscure.

How well do the various theories of food intake regulation account for the intermittent hunger and satiety that punctuate man's waking hours? It has already been pointed out how the glucostatic theory can account for this kind of periodicity. A lipostatic theory probably is incapable of accounting for the cyclical nature of hunger and satiety since such cycles can occur on a fat-free diet; moreover, the biochemical evidence strongly suggests that the rate of utilization of ingested and depot fat depends largely upon the availability of carbohydrate, not vice versa.

The thermostatic theory is believed by some investigators to explain the periodic nature of hunger and satiety. Booth and Strang[31] showed more than twenty years ago that the rise in skin temperature after eating has the same course in time as that of the specific dynamic action and correlates well with the onset of satiety. Preliminary studies by Strominger and Brobeck[32] suggest that the thermostatic explanation can circumvent some of the difficulties of the "one-nutrient" hypotheses. However, further work is needed to validate the thermostatic formulation.

A "liponeurostatic" theory based on the notion of neural connections between adipose cells and brain would not seem to be able to account for the cyclical nature of hunger and satiety, unless one believes that the hypothalamus can detect exceedingly small changes in the fat depots.

Finally, how can one account for the fact that prolonged satiety can be achieved on a carbohydrate-free diet?[33,34] It is known from the history of the use of pemmican (half and half, by weight, rendered fat and dehydrated lean) for food in the pioneer Northwest and in Arctic exploration that apparently normal satiety can occur on a diet free of carbohydrate.[34] Admiral Peary[35] has written as follows:

> "Too much cannot be said of the importance of pemmican to a polar expedition. It is an absolute sina qua non . . . With pemmican, the most serious sledge journey can be undertaken and carried to a successful issue in the absence of all other foods. . . . It is the most satisfying food I know."

It is not known whether appreciable fluctuations in blood glucose or glucose utilization can occur on a carbohydrate-free diet. Mellinkoff[37] has demonstrated that transient increases in peripheral uptake of glucose take place when amino acid mixtures are administered intravenously to human subjects. There is, as yet, no evidence that rates of glucose release from the liver on a carbohydrate-free diet will correspond to hunger-satiety patterns. It is therefore difficult, in terms of the available information, to see how the glucostatic theory can explain the hunger-satiety cycles that seem to occur with carbohydrate-free meals. Conceivably, the ingestion of protein could affect satiety by virtue of an influence on carbohydrate metabolism.

COMPLEXITY OF THE PROBLEM

It will be noted that in this discussion the complex role played by the digestive tract and particularly the

stomach in regulating hunger and satiety has not been emphasized. It is known that the stomach is dispensable as far as the control of food intake is concerned, and it seems well established that gastrointestinal factors, while important, are secondary in the energy balance scheme.[88]

We have not explained how any signal based on a constituent or effect of a meal can be translated so as to provide quantitative information to the regulatory center about calories consumed or the degree to which caloric requirements have been met. It is difficult to conceive of an immediate precise regulation of calorie intake. The body seems to maintain its composition by a constant process of correction, and the time factors involved suggest that the stimuli that signal satiety after a given meal are not necessarily the same ones that regulate body composition.

Indeed, we are left with the impression that, in terms of current knowledge, no one theory can encompass even the three simple exponible observations set forth earlier in the discussion. To explain the phenomena of hunger and satiety and the regulation of body composition a combination of known or suspected mechanisms may have to be invoked.

At any rate, there is growing evidence that the most productive investigations into the problem of food intake regulation are those which make use of technics from a variety of fields; for example, nutrition, physiology, biochemistry, and psychology. Such a multi-disciplinary approach seems well justified by the complexity of the problem.

SUMMARIO IN INTERLINGUA

Aspectos Physiologic De Fame E Satietate

Es discutite le problema del mechanismos physiologic que es responsabile pro le regulation del balancia de energia in le corpore. Es summarisate le historia del identification—per experimentos animal—de centros hypothalamic functionante como regulatores del ingestion de nutrimento. Es delineate e criticate le varie theorias que tenta explicar le maniera per que le centros hypothalamic recipe signales relative al stato de alimentation e de composition biochimic del organismo total. Le complexitate del problema es illustrate per referentias al cyclicitate del phenomenos de fame e satietate (que non reflecte directmente le requirimentos immediate del corpore). Le autor conclude que nulle theoria individual suffice pro explicar le phenomenos de fame e satietate e le regulation del composition del corpore e opina que un combination de cognoscite e non ancora cognoscite mechanismos participa in le situation total. Ille suggere correspondentemente que futur investigationes del problema procede per combinar e interrelationar technicas disveloppate in varie disciplinas interessate, i.e., per exemplo in le nutritiologia, le physiologia, le biochimia, e etiam le psychologia.

REFERENCES

[1] Lusk, G.: The Elements of the Science of Nutrition, ed. 2, Philadelphia, W. B. Saunders Company, 1909, p. 17.

[2] Brobeck, J. R., Tepperman, J., and Long, C. N. H.: Experimental hypothalamic hyperphagia in the albino rat. Yale J. Biol. & Med. *15*:831, 1943.

[3] Anand, B. K., and Brobeck, J. R.: Hypothalamic control of food intake in rats and cats. Yale J. Biol. & Med. *24*:123, 1951.

[4] Delgado, J. M. R., and Anand, B. K.: Increase of food intake induced by electrical stimulation of the lateral hypothalamus. Am. J. Physiol. *172*:162, 1953.

[5] Mayer, J.: Regulation of energy intake and the body weight; the glucostatic theory and the lipostatic hypothesis. Ann. N.Y. Acad. Sci. *63*:15, 1955.

[6] Skinner, B. F.: The Behavior of Organisms. New York, D. Appleton-Century, 1938.

[7] Miller, N. E.: Shortcomings of food consumption as a measure of hunger; results from their behavioral techniques. Ann. N.Y. Acad. Sci. *63*:141, 1955.

[8] Anliker, J., and Mayer, J.: The regulation of food intake. Some experiments relating behavioral, metabolic and morphologic aspects. Am. J. Clin. Nutrition *5*:148, 1957.

[9] Teitelbaum, P., and Campbell, B. A.: Ingestion patterns in hyperphagic and normal rats. J. Comp. Physiol. Psychol. *51*:135, 1958.

[10] Teitelbaum, P.: Random and food-directed activity in hyperphagic and normal rats. J. Comp. Physiol. Psychol. *50*:486, 1957.

[11] Miller, N. E., Bailey, C. J., and Stevenson, J. A .F.: Decreased "hunger" but increased food intake resulting from hypothalamic lesions. Science *112*:256, 1950.

[12] Durnin, J. V. G. A.: The day-to-day variation in individual food intake and energy expenditure. J. Physiol. *136*:34, 1957.

[13] Newman, R. W.: Skinfold measurements in young American males, in Brozek, J.: Body Measurements and Human Nutrition. Detroit, Wayne University Press, 1956, p. 47.

[14] Bernstein, L. M., and Grossman, M. I.: An experimental test of the glucostatic theory of regulation of food intake. J. Clin. Invest. *35*:627, 1956.

[15] Janowitz, H. D., and Hollander, F.: The time factor in the adjustment of food intake to varied caloric requirement in the dog: A study of the precision of appetite regulation. Ann. N.Y. Acad. Sci. *63*:56, 1955.

[16] Van Itallie, T. B.: Combined Staff Clinic. Obesity. Am. J. Med. *19*:111, 1955.

[17] Mellinkoff, S.: Digestive system. Annual Rev. Physiol. *19*: 195, 1957.

[18] Kennedy, G. C.: The role of depot fat in the hypothalamic control of food intake in the rat. Proc. Roy. Soc., London, s.B. *140*:578, 1952.

[19] Hausberger, F. X.: Über die innervation der fettorgans. Z. mikroskop.-anat. Forsch. *36*:251, 1934.

[20] Wertheimer, E., and Shapiro, B.: The physiology of adipose tissue. Physiol. Rev. *28*:451, 1948.

[21] Mayer, J.: Glucostatic mechanism of regulation of food intake. New England J. Med. *249*:13, 1953.

[22] Fredrickson, D. S., and Gordon, R. S., Jr.: Transport of fatty

acids. Physiol. Rev. *38*:585, 1958.

[23] Van Itallie, T. B., Beaudoin, R., and Mayer, J.: Arteriovenous glucose differences, metabolic hypoglycemia and food intake in man. J. Clin. Nutrition *1*:208, 1953.

[24] Stunkard, A. J., Van Itallie, T. B., and Reiss, B. B.: Effect of glucagon on gastric hunger contractions in man. Proc. Soc. Exper. Biol. & Med. *89*:258, 1955.

[25] Stunkard, A. J., and Wolff, H. G.: Studies on the physiology of hunger. I. The effect of intravenous administration of glucose on gastric hunger contractions in man. J. Clin. Invest. *35*:954, 1956.

[26] Duner, H.: The influence of the blood glucose level on the secretion of adrenalin and noradrenalin from the suprarenal. Scandinav. *28*(Suppl. 102):1, 1953.

[27] Bachrach, W. H.: Action of insulin hypoglycemia on motor and secretory functions of the digestive tract. Physiol. Rev. *33*:566, 1953.

[28] Grossman, M. I.: Integration of current views on the regulation of hunger and appetite. Ann. N.Y. Acad. Sci. *63*:76, 1955.

[29] Keys, A., Brozek, J., Henschel, A., Mickelsen, O., and Taylor, H. L.: The Biology of Human Starvation. Volume I. Minneapolis, University of Minnesota Press, 1950, p. 273.

[30] Teitelbaum, P.: Sensory control of hypothalamic hyperphagia. J. Comp. Physiol. Psychol. *48*:156, 1955.

[31] Booth, G., and Strang, J. M.: Changes in temperature of the skin following the ingestion of food. Arch. Int. Med. *57*:533, 1936.

[32] Strominger, J. L., and Brobeck, J. R.: A mechanism of regulation of food intake. Yale J. Biol. & Med. *25*:383, 1953.

[33] Lieb, C. W.: The effects on human beings of a twelve months' exclusive meat diet. J.A.M.A. *93*:20, 1929.

[34] Stefansson, V.: The Fat of the Land. New York, MacMillan, 1957, p. 178.

[35] Peary, R. E.: Secrets of Polar Travel. New York, Century Company, 1917, p. 78.

[36] Mellinkoff, S. M., Frankland, M., Boyle, D., and Greipel, M.: Relationship between serum amino acid concentration and fluctuations in appetite. J. Appl. Physiol. *8*:535, 1956.

[37] Mellinkoff, S. M., Frankland, M., and Greipel, M.: Effect of amino acid and glucose ingestion on arteriovenous blood sugar and appetite. J. Appl. Physiol. *9*:85, 1956.

[38] Janowitz, H. D.: Editorial. Hunger and appetite. Physiologic regulation of food intake. Am. J. Med. *25*:327, 1958.

Metabolism of Essential Fatty Acids

Linoleic, linolenic and arachidonic acids are the three most widely recognized members of the group of higher polyunsaturated fatty acids exhibiting essential fatty acid activity. However, the biological activity of linolenic is known to differ considerably from that of linoleic and arachidonic acids (*Nutrition Reviews* *10*:154, 1952), which suggests that the metabolic fate of linolenic acid is different from that of the other two fatty acids. On the assumption that information about the metabolism of linoleic acid is necessary in order to explain these differences in biological activity, G. Steinberg, W. H. Slaton, Jr., D. R. Howton and J. F. Mead (*J. Biol. Chem.* *224*:841, 1957) have studied the fate of isotopically labeled linoleic acid in the animal body.

About 90 mg. of a mixture containing 40 per cent of methyl linoleate-1-C^{14} and 60 per cent of corn oil were administered to each of a group of ten rats. Eight of the ten were killed after four hours. The other two were retained for respiratory carbon dioxide estimations. The livers, kidneys, hearts, spleens and abdominal fat of the animals were pooled and the higher unsaturated fatty acids were isolated as insoluble polybromides. These were debrominated, hydrogenated and separated by reversed phase chromatography. The arachidic acid (C_{20}) obtained was degraded in such a way that the radioactivity of each of the four carbon atoms, beginning with the carboxyl carbon, could be determined separately. The radioactivity of the carboxy-carbon of the stearic acid was also determined. The linoleic acid was isolated and purified and a fraction of the higher polyene mixture was isomerized.

As much as 30 per cent of the carboxy carbon of linolenic acid was recovered in the respiratory CO_2. However, the low value of 6 per cent for one rat indicates the variability that may be encountered. The measurement of radioactivity in the various lipid fractions isolated indicated that the carboxyl carbon from linolenic acid had been incorporated into the fatty acids of the saturated and various unsaturated fractions.

In sequence of steps, degradation of the C_{20} acids isolated from the unsaturated fatty acid fraction yielded the following information: The carboxyl carbon contained 27 per cent of the total radioactivity present in the C_{20} fraction and, counting from the carboxyl end, C_2 contained 8 per cent; C_3, 60 per cent; C_4, 4 per cent and the 16 carbon residue, 1 per cent. A comparison of the distribution of the labeling in the C_{18} acids isolated from the unsaturated fatty acid fraction with that in the original linolenate revealed that there was labeling beyond the original label in the carboxyl carbon, thus indicating that the labeling in C_4 and in the C_5 to C_{20} chain was real. A similar distribution pattern was found in the carbon skeleton of the polyene mixture. It appeared from these results that the linolenate had undergone several β-oxidations without affecting the double bond system and that the molecule was rebuilt from acetate, some of which arose from β-oxidation of the labeled linolenate. In similar experiments in which carboxy-labeled linoleate was used no radioactivity was detected beyond carbon atom 3 of the C_{20} acids (Steinberg, Slaton, Howton and Mead, *Ibid.* *220*:257, 1956).

From *Nutrition Reviews,* Vol. 16, No. 2, pp. 55-56.

EDITORIAL

HUMAN GROWTH HORMONE

The earliest knowledge of the function of the adenohypophysis was related to its importance in human growth. At the time of Marie's description of acromegaly in 1886 the nature and function of the pituitary was not known. The enlargement of the pituitary in acromegaly and in gigantism drew attention to the organ and led to the first appreciation of its function. An elementary understanding of the gland was evident in Hutchinson's statement in 1900, that "in the pituitary body we appear to have a sort of growth-regulating centre for the entire body, the disturbance of which in early life will produce the phenomena of giantism, and in later life those of acromegaly."[1] Subsequently the pituitary was shown to contain an anabolic hormone which promoted growth, and ways of purifying the growth hormone were devised. The complex pattern of metabolic effects produced by this hormone was studied extensively in animals, but only in the past two years, through the use of human growth hormone, were reproducible effects obtained in man.

The preparation of growth hormone from human pituitaries was undertaken after many failures of material from slaughterhouse animals to prove satisfactory in human studies and following suggestions from animal studies of species differences among growth hormones.[2,3] Pituitaries obtained at autopsy and stored for months in acetone proved to be a satisfactory source of human growth hormone.[4] When prepared by the glacial acetic acid method[5] the human growth hormone preparation was regularly active in man and appeared to be antigenically identical with the hormone present in human blood.[6] Anabolic effects were observed in balance studies by Beck, Pearson, Henneman and their associates[7-9] and similar effects of a preparation made by an alkaline extraction procedure[10] were reported by Bergenstal[11] and by Ikkos.[12] Storage of nitrogen, phosphorus and potassium was usually observed, while effects on calcium and sodium balance were more erratic. Urinary excretion of calcium was frequently increased even when the overall balance was positive. Nitrogen retention was produced with a dose of 1 mg./day in a hypopituitary subject and 2.5 mg./day in an adult subject with normal pituitary function. The initial effect on nitrogen was often large, with storage of 3 or 4 gm. of nitrogen per day. There was usually a fall in blood urea nitrogen concentration within twenty-four hours of a single injection of human growth hormone.[13]

Anabolic doses of human growth hormone in the range of 30 to 150 μg./kilogram/day had little or no effect on blood sugar, glucose tolerance or blood ketones in normal individuals and in nondiabetic hypopituitary subjects[14,15] but a diminished hypoglycemic response to insulin was observed in two of the hypopituitary group.[15] Glucose tolerance was impaired in a nondiabetic pituitary dwarf receiving 600 μg./kilogram/day.[7] Hypophysectomized diabetic patients receiving insulin were more sensitive to diabetogenic effects, exhibiting ketosis and sometimes increased hyperglycemia while receiving approximately 75 to 150 μg./kilogram/day.[15,16]

Growth hormone was found to increase the unesterified fatty acids of plasma[17] and perhaps this effect of growth hormone, by increasing the available substrate for ketone formation, accounts for its ketogenic action. Growth hormone of porcine, bovine, simian and human origin raised fatty acids in fasting dogs, but only human and simian preparations did so in man. The fasting value in man was usually doubled or tripled within four hours by human growth hormone. The effect was suppressed by glucose and food in nondiabetic subjects, but not in the uncontrolled diabetic. The pattern of response of fatty acids to growth hormone suggested that the hormone might normally influence the rate of fat mobilization in the postabsorptive period.[18] Whether fat mobilization is directly facilitated by the hormone or is a response to a need for more calories occasioned by the anabolic effects of the hormone is not known. However, the increased provision of calories from adipose tissue undoubtedly contributes to the anabolic result, and may permit, as in a study of an obese subject,[19] growth hormone to induce a large storage of nitrogen in a subject on a calorically inadequate diet.

The prolonged use of human growth hormone was well tolerated and was effective in stimulating growth. Two milligrams intramuscularly three times a week produced in a pituitary dwarf a growth rate of 2.6 in. per year, and an increase in serum inorganic phosphorus and alkaline phosphatase.[20] There was no change in blood sugar and no allergic reactions in twenty months of treatment.

While apparently not antigenic in man, human growth hormone induced the formation of antibodies in rabbits, permitting Read and Stone to devise an immuno-

logical method for the estimation of the hormone in human blood.[6]

The name growth hormone is likely to suggest that the function of the hormone is limited to the period of growth and to obscure the possibility that this most anabolic of agents may be physiologically important throughout life. Although the opportunity to evaluate growth hormone in human physiology and disease has only recently become possible, it has been seen that man continues to secrete growth hormone in adult life,[6,21] and that the mature and even the aged exhibit metabolic effects from administered human growth hormone.[18] The availability of human growth hormone promises to open up a field of clinical investigation of wide scope and interest.

REFERENCES

[1] Hutchinson, W.: The pituitary gland as a factor in acromegaly and giantism. N. Y. Med. J. 72:133, 1900.

[2] Wilhelmi, A. E.: The Hypophyseal Growth Hormone: Nature and Actions. R. W. Smith, Jr., O. H. Gaebler, and C. N. H. Long, Eds., New York, Blakiston, 1955, p. 59.

[3] Knobil, E., Wolf, R. C., and Greep, R. O.: Some physiological effects of primate pituitary growth hormone preparations in the hypophysectomized rhesus monkey. J. Clin. Endocrinol. & Metab. *16*:916, 1956.

[4] Raben, M. S.: Preparation of growth hormone from pituitaries of man and monkey. Science *125*:883, 1957.

[5] Raben, M. S., and Westermeyer, V. W.: Recovery of growth hormone in purification of corticotropin. Proc. Soc. Exp. Biol. & Med. *78*:550, 1951.

[6] Read, C. H., and Stone, D. B.: An immunologic assay for minute amounts of human pituitary growth hormone. A.M.A. J. of Dis. of Child. *96*:538, 1958.

[7] Beck, J. C., McGarry, E. E., Dyrenfurth, I., and Venning, E. H.: Metabolic effects of human and monkey growth hormone in man. Science *125*:884, 1957.

[8] Pearson, O. H., Lipsett, M. B., Greenberg, E., and Ray, B. S.: Effects of human growth hormone in hypophysectomized patients. Abstract No. 40, Endocrine Soc. Meeting, New York, 1957.

[9] Henneman, P. H., Moldawer, M., Carroll, E. L., Mitchell, M., and Waddell, M. J.: Human growth hormone: prolonged administration of moderate doses in three patients with panhypopituitarism. Abstract No. 40, Endocrine Soc. Meeting, San Francisco, 1958.

[10] Li, C. H., and Papkoff, H.: Preparation and properties of growth hormone from human and monkey pituitary glands. Science *124*:1293, 1956.

[11] Bergenstal, D. M., Lubs, H. A., Hallman, L. F., Patten, J., Levine, H. J., and Li, C. H.: Metabolic effects of human and monkey growth hormone in man. J. Lab. Clin. Med. *50*: 791, 1957.

[12] Ikkos, D., Luft, R., and Gemzell, C. A.: The effect of human growth hormone in man. Lancet *1*:720, 1958.

[13] Raben, M. S., and Hollenberg, C. H.: Assay of growth hormone for activity in man. Abstract No. 39, Endocrine Soc. Meeting, San Francisco, 1958.

[14] Henneman, Dorothy H.: Pattern of carbohydrate and fat metabolism during prolonged effective doses of human growth hormone in man. Abstract No. 70, Endocrine Soc. Meeting, San Francisco, 1958.

[15] Greenberg, E. J., Grayzel, J., Ray, B. S., and Pearson, O. H.: Influence of human growth hormone on carbohydrate metabolism in man. Clin. Res. *6*:242, 1958.

[16] Luft, R., Ikkos, D., Gemzell, C. A., and Olivecrona, H.: Effect of human growth hormone in hypophysectomized diabetic subjects. Lancet *1*:721, 1958.

[17] Raben, M. S., and Hollenberg, C. H.: Effect of growth hormone on plasma fatty acids. J. Clin. Invest. *37*:922, 1958.

[18] Raben, M. S.: Human growth hormone in Recent Progress in Hormone Research. G. Pincus, Ed. New York, Academic Press, Vol. 15, in press.

[19] Henneman, P. H.: Personal communication, 1958.

[20] Raben, M. S.: Treatment of a pituitary dwarf with human growth hormone. J. Clin. Endocrinol. & Metab. *18*:901, 1958.

[21] Read, C. H.: Personal communication, 1958.

M. S. RABEN, M.D.
Boston

BOOK REVIEW

INSULIN CRYSTALS. *By Jørgen Schlichtkrull. d. kr. 30 ($4.35), pp. 140, Ejnar Munksgaard, Publishers, Copenhagen, 1958.*

The studies described in Schlichtkrull's monograph were initiated in 1950 in conjunction with other investigators in the Novo Laboratories in Copenhagen. In the succeeding years this team systematically investigated various aspects of the chemical, biological, and clinical factors influencing the relationship of zinc and insulin, and uncovered much new knowledge about the timing of insulin suspensions. They discovered that long-acting preparations could be prepared without the addition of foreign protein modifying agents, a finding which eventuated in the development of Lente insulin preparations. The present report extends still further these studies of the physicochemical factors involved in crystal suspensions containing no added zinc ions, but which do show a protracted action. When ordinary beef insulin crystals containing two atoms of zinc per unit-cell (or more) were subjected to the influence of temperature under certain conditions, the time action was prolonged. Urea, formamide, and halide in the medium resulted in the formation of sharp-edged single-rhombohedrons having still more prolonged effects. A variety of timings may be produced, but it is not yet known whether these preparations are useful clinically, or whether they have any advantages in therapy.

The monograph is attractively printed and illustrated. The author has succeeded in presenting highly technical data in an understandable manner and he has included background summaries of previous work which are documented by the important bibliographical references. His illustrations of crystal structure are particularly noteworthy. The work represents a significant contribution to knowledge in a field that is not widely followed.

Raoul Boulin

Paul Rambert, M.D., Paris

Raoul Boulin, distinguished French physician and student of diabetes, died Aug. 1, 1958, at the age of sixty-five. Hardly halfway through his medical studies when the first World War broke out, he served as Battalion Medical Officer in a shock division and was mentioned in dispatches several times. In 1920 he passed the competitive medical examination and quickly obtained the highest University and Hospital degrees from Paris University. Appointed "Médecin des Hopitaux" of Paris in 1928, he passed the agrégation (University staff) examination in 1933. In 1954 his work in metabolic disorders was acknowledged by the creation of a special Chair in Paris University devoted to diabetes and nutritional diseases, we believe the very first in the world. He was Honorary President of the French Diabetes Association at the time of his death.

In the course of his medical studies, he worked under Widal, Ravaut and Marcel Labbé. While serving with Labbé at the Medical Clinic at the Hospital Cochin, he began his clinical research.

At an earlier stage, he had published with Ravaut remarkable papers on the modifications of the cerebrospinal fluid and on Nicolas-Favre disease.

Highly intelligent, and possessing great intellectual honesty, Raoul Boulin left an imperishable mark, based on rigorous observation and care, not only of the sick people in this department, but also of the great many private patients whom he regularly visited. He created at the Hospital de la Pitié one of the most active diabetic welfare centers in the world, which annually attracted more than 9,000 patients in consultation. Under his care nearly 100 acidotic patients in coma were treated. Thanks to the well-trained medical team he developed, the rate of mortality due to coma was only 2 per cent.

He wrote more than 400 articles on diabetes. Among his most important contributions, we may note his outstanding work on hemochromatosis, its unelaborate forms, the endocrine and metabolic disorders it provokes, and its familial nature. He more particularly studied the paradiabetic complications and invented the valuable provocative hyperglycemia test which he detailed with Marcel Labbé. In his last years, he gave a new impulse to research in the prevention of diabetes among predisposed subjects and created a special consultation department for their care.

He also particularly studied the treatment of diabetic coma, its renal and hepatic complications, and especially the electrolytic disturbances. Having witnessed the first use of insulin, he devoted numerous articles to the various long-acting insulins; in his last paper he reported his experiments with the hypoglycemic sulfonamides on 200 diabetic subjects studied for more than two years.

He was especially interested in diabetes of children and adolescents and used to insist upon their need for proper diet.

France and medicine generally have lost a distinguished physician and student of diabetes. He was one of the most famous French consultants in diabetes, and his pupils in France as well as abroad are numerous. Always courteous toward everyone, ignoring anything which could look like intrigue, he was highly esteemed by all. He was most interested in the work of his foreign colleagues and always looked forward to meeting them at the International Congresses. He had special respect for Dr. Elliott Joslin, whose friendship he had enjoyed and cherished for many years.

Grow old along with me; the best is yet to be,
The last of life for which the first was made.
Our times are in His hand, who saith
A whole I planned. Youth shows but half;
Trust God, see all, nor be afraid.

By Robert Browning in *Rabbi Ben Ezra*
Houghton Mifflin Company.

Since, of course, the metabolism of carbohydrates, fats, and proteins is closely integrated through the tricarboxylic acid cycle, it is well within the range of possibility that before too long the entire metabolic processes of man may be diagrammed as a single, elaborate biochemical pattern. Through the continuing study of more and more mutant individuals, each enzyme in the pattern will become identifiable in terms of the activity of a specific gene (a specific portion of a deoxyribonucleic acid molecule), and each genetic enzyme dysfunction will be related either to a mutation of that gene, resulting in a lack or modification of the enzyme, or to a mutation of a different gene, acting in such a way as to inhibit the activity of the enzyme.

Laurence H. Snyder, in "Fifty Years of Medical Genetics," from *Science,* Jan. 2, 1959.

"Diabetes—Today and Tomorrow:
The Expanding Role of the Doctor"

Diabetes exhibit will be displayed at annual meeting of American Medical Association.

The American Diabetes Association will display an exhibit entitled "Diabetes—Today and Tomorrow: The Expanding Role of the Doctor," at the annual meeting of the American Medical Association June 8-12 in Atlantic City.

The exhibit is intended to give physicians a greater understanding of their own important role in the care of their diabetic patients. It is pictured above in its entirety, and detailed close-ups of its salient features appear on the following two pages.

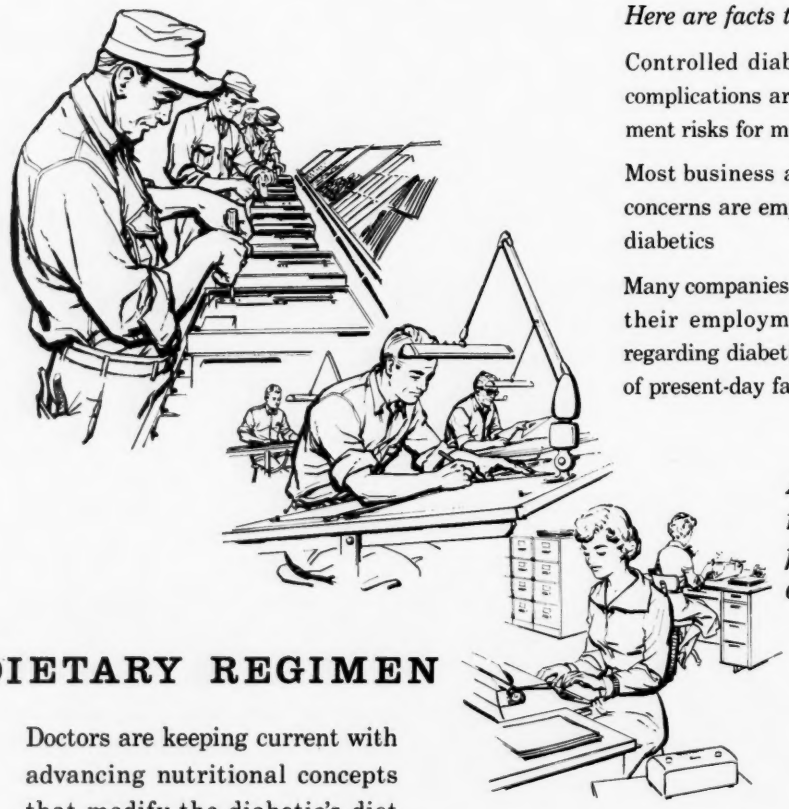
For instance, to illustrate the advancing concepts of nutrition and how they affect the dietary regimen of the diabetic, a chart has been prepared to show how percentage values of total calories in diet from fat, carbohydrate, and protein have changed in the past thirty years. A forecast for the future shows that the percentage of calories from fat will continue to shrink, but that the diabetic will still need a planned diet.

In the field of hypoglycemic agents, various oral compounds are examined, with particular emphasis on their effectiveness with special types of diabetic patients. A chart shows the comparative performances of seven varieties of insulin.

The exhibit shows the physician his own special role in the program for employment of diabetics. First of all, it depicts how he can help the diabetic by educating him to live with diabetes and keep fit for work; then how he can help management and labor further their understanding of diabetes; and then how he can help to encourage insurance coverage for all diabetic employees.

Planning and development were under the direction of the ADA Committee on Scientific Exhibits, of which Marshall I. Hewitt, M.D., is Chairman. Committee members include DeWitt E. DeLawter, M.D., Robert C. Hardin, M.D., William R. Kirtley, M.D., Leo P. Krall, M.D., C. J. O'Donovan, M.D., and George F. Schmitt, M.D.

"Diabetes—Today and Tomorrow: The Expanding Role of the Doctor" will be added to the ADA scientific exhibits already available on a loan basis to Clinical Societies of Affiliate Associations and to medical organizations. Other exhibits are: "Pathology of Diabetes," "Management of Diabetes Mellitus," "Vascular Complications of Diabetes," and "Diabetes Detection by the Physician."



Here are facts to emphasize:

Controlled diabetics without complications are good employment risks for most occupations

Most business and industrial concerns are employing known diabetics

Many companies need to review their employment practices regarding diabetics in the light of present-day facts

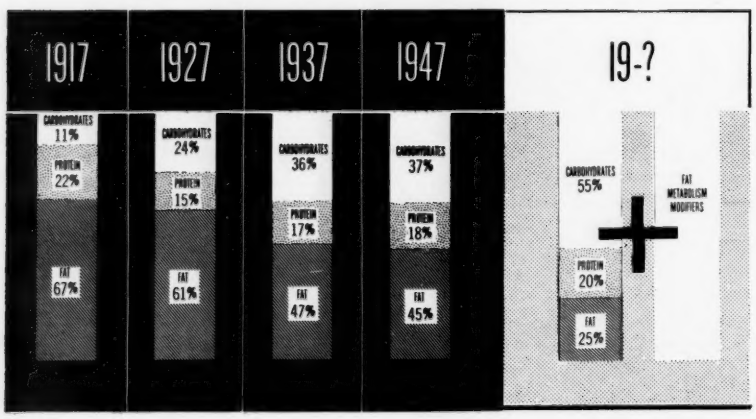
A fuller and more productive life for all employable diabetics

A work force strengthened by diabetics in suitable jobs

DIETARY REGIMEN

Doctors are keeping current with advancing nutritional concepts that modify the diabetic's diet

Here's how the percentage of total calories from fat, carbohydrate and protein have changed in the past three decades:



A FORECAST FOR TOMORROW

The percentage of calories from fat will continue to shrink, and fat metabolism modifiers may be added

A planned diet for every diabetic will still be needed

HYPOGLYCEMIC AGENTS

Present-day oral hypoglycemic agents are not true insulin substitutes.

Doctors are keeping current with the latest developments that affect the diabetic's treatment

No one insulin is the answer to the needs of every diabetic

Not all diabetics show the same hypoglycemic effect from the same type of insulin

1-(*P*-METHYLPHENYLSULFONYL)-3-BUTYLUREA (TOLBUTAMIDE)

1. Most effective in relatively mild adult diabetes using up to moderate amounts of insulin
2. Contraindicated in diabetics with:
   a. Onset in childhood or adolescence
   b. Unstable diabetes
   c. History of coma
   d. Most surgery
   e. Ketosis, acidosis, infection, severe trauma

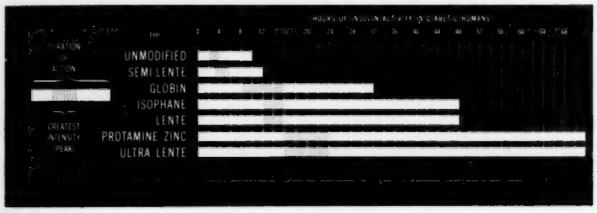
1-PHENETHYLBIGUANIDE (OR ANALOGS)

1. Reduces hyperglycemia and glycosuria in many diabetics
2. G-I symptoms in fairly high percentage
3. No evidence of systemic toxicity
4. Published data insufficient to clarify present and future use

1-(*P*-CHLOROBENZENESULFONYL)-3-N-PROPYLUREA (CHLORPROPAMIDE)

1. Most effective in relatively mild adult diabetes using up to moderate amounts of insulin
2. Contraindications the same as for tolbutamide
3. Its action because of slower excretion is more prolonged than tolbutamide, requiring greater care to prevent hypoglycemia and side effects
4. Patients unresponsive to tolbutamide may respond to chlorpropamide, and vice versa

PATIENT RESPONSE TO EACH TYPE OF INSULIN



The quest will continue for the theoretic "automatic" insulin

The search will continue for the theoretic ideal oral agent- a true insulin substitute free from side effects in acute and chronic use

EMPLOYMENT

*A recent survey of industry shows:**

69% of companies surveyed employ known diabetics

94% report better or average work records for diabetics

56% which do not employ known diabetics keep them on when diabetes develops after they are hired

51% of medical departments do routine laboratory tests for diabetic employees

* Representing 127 firms with 1,871,640 employees. from Beardwood J. T. Jr. et al: Analysis of a Survey Concerning Employment of Diabetics, in Report of Committee on Employment, American Diabetes Association, Inc. Diabetes 6: 550-554 (Nov.-Dec.) 1957

Here's what doctors are doing to aid the employable diabetic:

FOR THE DIABETIC HIMSELF:

Help him to live with diabetes ...and keep fit for work

FOR MANAGEMENT AND LABOR:

Further their understanding of the diabetic employee

FOR INSURANCE COMPANIES:

Encourage coverage for diabetic employees

ABSTRACTS

Allwood, M. J.; Birchall, I.; and Staffurth, J. S. (The Sherrington Sch. of Physiol. and St. Thomas's Hosp., London, S.E. 1, England): CIRCULATORY CHANGES IN THE FOREARM DURING INSULIN HYPOGLYCAEMIA STUDIED BY REGIONAL ^{24}NA CLEARANCE AND BY PLETHYSMOGRAPHY. J. Physiol. *143*: 332-42, Sept. 23, 1958.

Blood flow in the human forearm was measured by plethysmograph concurrently with clearance of Na^{24} from the opposite forearm, both before and during insulin hypoglycemia. Average blood sugar levels before and during insulin hypoglycemia were 92 and 32 mg. per cent, respectively. The Na^{24} was injected either intradermally or intramuscularly as an isotonic sodium chloride solution. Forearm blood flow increased during hypoglycemia, as did Na^{24} clearance from forearm *skin*. Changes in isotope clearance of Na^{24} from forearm *muscle* at this time were not significant. Blockade of cutaneous nerves with 2 per cent lignocaine did not influence the resting clearance of Na^{24} from nerve-blocked skin composed with that from the opposite limb serving as a control. During insulin hypoglycemia there was an increase in Na^{24} clearance in the untreated arm in most experiments, but this did not occur in the nerve-blocked arm. It is concluded that part of the increased blood flow which occurs in the intact forearm during insulin hypoglycemia is located in the cutaneous blood vessels which are normally under little or no vasoconstrictor tone. The increased skin flow during hypoglycemia in the normal subject is mediated by vasodilator fibers in cutaneous nerves. G.A.W.

Annino, Joseph S.; and Relman, Arnold S. (Massachusetts Memorial Hospitals, Boston, Mass.): THE EFFECT OF EATING ON SOME OF THE CLINICALLY IMPORTANT CHEMICAL CONSTITUENTS OF THE BLOOD. Am. J. of Cl. Path. *31*:155-59, March 1959.

This study gives a comparative analysis of blood specimens for most diagnostic chemical analyses in the fasting and postprandial state. Thirty-two normal subjects were tested. It is concluded that the normal person with an average breakfast does not significantly alter the blood constituents with the exception of glucose and phosphorus. R.W.S.

Banner, Edward A.; and Beutler, Herbert K. (Sect. of Obst. and Gynec., Mayo Clin. and Mayo Foundation, Rochester, Minn.): INTRAUTERINE DEATHS. Obst. & Gynec. *12*:661-69, December 1958.

A study has been made of 190 intrauterine deaths, as recorded at the Mayo Clinic during a ten-year period. Each death followed a definite diagnosis of intrauterine life. The principal causes of these deaths are listed. Almost all fetuses will deliver within two weeks after intrauterine death. Induction of labor is usually not necessary, but arrangements should be made for replacement of excessive blood loss. Of the patients in whom intrauterine death of a fetus is caused by abruptio placentae, approximately 60 per cent will require blood replacement. Early delivery after control of toxemia seems to be the only hope when severe toxemia develops in the latter weeks of pregnancy. Primigravidas especially seemed to be susceptible to the development of early toxemia and intrauterine death. Intrauterine death among women with severe diabetes is not uncommon. Usually it occurs between the thirty-fourth and thirty-sixth week of pregnancy. Most infants, at this stage, are of average weight. The diabetic age of these mothers is generally high. The length of time a dead fetus is retained in utero does not necessarily influence the degree of postpartum hemorrhage sustained by the mother. E.A.W.

Bastenie, P. A.; and Szucs-Verbiest, M. (Dept. of Med., St. Pierre Hosp., Univ. of Brussels, Brussels, Belgium): RELATIONSHIP BETWEEN GLUCOSE UPTAKE AND CHANGES IN INORGANIC SERUM PHOSPHORUS. Metabolism *VIII*:114-19, March 1959.

The authors review changes in inorganic serum phosphorus following the rapid intravenous injection of glucose in normal and diabetic subjects. In patients treated with insulin, cortisone, or ACTH in a variety of diseases, the rate of disappearance of glucose was compared to the maximum drop in inorganic phosphorus. The normal subjects, the diabetics, and the insulin-treated normal subjects showed marked difference in the values for both glucose assimilation and drop in inorganic phosphorus. Treatment with cortisone induced slight changes in glucose uptake without correlating changes in serum phosphorus. The authors concluded that changes in inorganic serum phosphorus which follow the intravenous injection of glucose yield no specific diagnostic information in the study of carbohydrate metabolic alterations. R.W.S.

Bing, R. J.; Choudhury, J. D.; Michal, G.; and Kako, K. (Dept. of Med., Washington Univ. and the Washington Univ. Med. Serv., VA Hosp., St. Louis, Mo.): MYOCARDIAL METABOLISM. Ann. Int. Med. *49*:1201-15, November 1958.

This is a review of recent work on the metabolism of the beating and arrested heart in the normal and diseased (congestive failure, coronary occlusion, diabetes) humans and animals. The heart from the diabetic mammal shows various biochemical defects in glucose, protein and fat metabolism, correctable by insulin administration. There is a decrease in the proportion of energy derived from carbohydrate sources. The latter contribute only 35 per cent to the normal myocardial oxidative process. The authors conclude, however, that this does not constitute a serious disturbance in the energy utilization of the heart. S.B.B.

Bloedow, Carman E. (Veterans Administration Hosp. and University of Oklahoma Hosps., Oklahoma City, Okla.): THE USE OF CHLORPROPAMIDE AS AN ORAL HYPOGLYCEMIC AGENT. J. Oklahoma M. A. *51*:655-58, November 1958.

A new sulfonylurea compound, chlorpropamide, has been found to be effective in the treatment of diabetes mellitus. The same criteria useful in the selection of patients for treatment with tolbutamide apply to those being considered for treatment with chlorpropamide. The elderly, mild, diabetic appears to be more prone to hypoglycemic reactions when treated with chlorpropamide than when treated with comparable doses of tolbutamide. The fasting blood glucose seems to reach a plateau during the sixth to the eleventh day after therapy

with chlorpropamide is initiated. The onset of action of chlorpropamide is approximately the same, but the duration of action is greater than that of tolbutamide. Chlorpropamide seems to be a more potent hypoglycemic agent than tolbutamide. This could be due to greater potency of chlorpropamide at equivalent blood levels, or to better absorption of chlorpropamide than tolbutamide, or to slower detoxification and excretion of chlorpropamide. B.F.K.

Bolinger, R. E.; van der Geld, H.; and Willebrands, A. F. (Second Medical Service, Wilhelmina Gasthuis, Amsterdam, The Netherlands): ELECTROPHORETIC SEPARATION OF INSULIN ACTIVITY OF NORMAL PLASMA AND PLASMA WITH ADDED INSULIN-I^{131}. Metabolism *VIII*:39-47, January 1959.

The authors present evidence demonstrating by electrophoretic separation that insulin is bound to the beta and gamma globulin fractions in normal human serum subjected to starch-column fractionation. The highest peak of insulin action appears in the beta fraction and none in the albumin fraction. Insulin-I^{131} added to normal serum demonstrated that radioactive and biological activity is associated with the beta fraction. A small quantity of radioactivity was noted in the alpha globulin. Insulin-I^{131} has the electrophoretic mobility of beta globulin. There is marked absorption of radioactive insulin to the paper. This can be prevented by presoaking the paper with a normal insulin solution but not with albumin or gamma globulin solution. This may explain the small amount of radioactive insulin attached to the alpha globulin.

These results indicate that insulin activity of normal human serum is due to the presence of insulin since the peak of biological activity corresponds to the peak of radioactivity when insulin-I^{131} is added. Since gamma globulin fraction of normal serum shows some biological activity, endogenous insulin within the plasma may be present in several states of combination with beta or gamma globulin but not with albumin and probably not with alpha globulin. R.W.S.

Bothe, Frederick A.; Beardwood, Joseph T., Jr.; Kirk, Robert D.; and Henderson, Theodore A. (Philadelphia, Pa.): THE SURGICAL DIABETIC. West. J. Surg. 66:215-19, July-August 1958.

The medical management of the diabetic requiring elective and emergency surgery is discussed. Emphasis is placed on the necessity to correct acidosis before surgery. Regular insulin is used conservatively to prevent the complications of a hypoglycemic reaction. The use of oral hypoglycemic agents is contraindicated. Conservative or radical surgery in gangrene of the feet depended upon the presence or absence of a collateral circulation. Long-standing diabetes was accompanied by rich collateral circulation and gangrene of the heel was invariably accompanied by thrombosis of the posterior tibial artery and required radical amputation. The incidence of malignancy, gangrene, cholecystitis and carbuncles was reported in a series of 5,224 diabetics. A.S.R.

Brozek, Josef; Mori, Hiroyoshi; and Keys, Ancel (Lab. of Physiological Hygiene, University of Minnesota, Minneapolis, Minn.): ESTIMATION OF TOTAL BODY FAT FROM ROENTGENOGRAMS. Science *128*:901, Oct. 17, 1958.

The authors present equations for predicting body density from roentgenographic measurements of the thickness of the subcutaneous fat. The measurements were obtained from soft-tissue roentgenograms of the upper arm, forearm, and calf. Total body fat was estimated from body density, the body volume being obtained by underwater weighing. Coefficients of correlation between body density and roentgenographic measurements are cited, based upon fifty-two middle-aged business and professional men as subjects. L.S.S.

Burt, Richard L. (Dept. of Obstet. & Gynec., Bowman Gray School of Medicine of Wake Forest College and North Carolina Baptist Hosp., Winston-Salem, N. C.): REACTIVITY TO TOLBUTAMIDE IN NORMAL PREGNANCY. Obstet. & Gynec. *12*:447-53, October 1958.

The author has studied the mechanism of action of tolbutamide in normal pregnancy and compared its action to insulin. He concluded that, as with insulin, resistance to the hypoglycemic effect of tolbutamide develops in pregnancy. Also, the data presented are consistent with the view that the sulfonylurea compounds produce at least part of their total metabolic effect by stimulation of insulin release. Lastly, the hypo-amino-acidemia developing after administration of tolbutamide is qualitatively comparable to that following insulin administration but is exaggerated in pregnancy. This finding is believed to be unrelated to the effect on blood sugar. E.A.W.

Creutzfeldt, W.; and Moench, A. (Dept. of Medicine, Univer. of Freiburg, Germany): COMPARATIVE STUDIES WITH THE HYPOGLYCEMIC GUANIDINE DERIVATIVES SYNTHALIN B AND PHENETHYLDIGUANIDE (DBI): A CONTRIBUTION TO THE PROBLEM OF THE SO-CALLED A-CELL POISONS. Endokrinologie 36:167-85, July 1958.

Synthalin B (dodecamethylenediguanidine) when given to the guinea pig for several days, causes marked degranulation and vacuolization of the Z cells in the islets of Langerhans, while the β cells remain unaltered. The α-cell changes are somewhat less marked than after administration of Synthalin A. Yet Synthalin A and B have about the same effect on extrapancreatic organs: The adrenal cortex appears markedly stimulated; the cells of the adrenal medulla show degranulation and vacuolization, particularly in animals with hypoglycemic shock; the liver cells show both unspecific diffuse and specific periportal damage; the kidney gives evidence of toxic nephrosis.

Phenethyldiguanide (DBI), although quite similar to the Synthalins in regard to its hypoglycemic effect, causes degenerative changes in the pancreatic α cells of the guinea pig of a far lesser degree than Synthalin and only irregularly. Its effect upon the adrenals, the liver and kidneys too is less marked, yet demonstrable. Large doses may cause degenerative changes in the liver and complete disappearance of the hepatic glycogen.

The observation that hypoglycemic action of these different guanidine derivatives is accompanied by quantitatively different changes in the pancreatic α cells is an argument against the concept that the α cell changes are the cause of the hypoglycemic reaction or that the guanidine preparations exert their hypoglycemic effect through an alphacytotoxic action. The observed α-cell changes are considered as results of functional exhaustion, probably secondary to liver damage (German). M.G.G.

DeMeutter, Roger C.; Khachadurian, Avedis K.; and Marble, Alexander (Cancer Res. Inst. and Baker Clin. Res. Lab., N. E. Deaconess Hosp., Boston, Mass.): IMMEDIATE EFFECTS OF INTRAVENOUS INJECTIONS OF TOLBUTAMIDE AND INSULIN ON BLOOD GLUCOSE AND AMINO ACIDS. Proc. Soc. Exper. Biol. & Med. 99:33-35, October 1958.

The authors determined that the intravenous injection of sodium tolbutamide in normal subjects in a dose of 40 mg./kg. produced hypoglycemia comparable to that produced by insulin in a dose of 1/10 U./kg. body weight. In three of five subjects tested the fall in blood glucose took place within five minutes following the injection of tolbutamide. In patients with stable diabetes, the decrease in blood glucose following tolbutamide was slower than that of insulin. In the normal individuals and in the diabetic patients who experienced a fall in blood glucose following the injection of tolbutamide, there was a decrease in serum amino acids comparable to that following the intravenous injection of insulin. G.J.H.

Editorial. BLOOD SUGAR IN CYCLIC VOMITING. Brit. M. J. 2:1585, Dec. 27, 1958.

The author comments on the differential diagnosis between cyclic or recurrent vomiting and diabetic coma. The former condition may be accompanied by hyperglycemia. Two other conditions to be differentiated are appendicitis and cerebral tumor. The author advises that a good physical examination helps immeasurably in resolving the differential diagnosis. C.A.R.

Editorial. THE DIABETIC SCHOOL-LEAVER. Brit. M. J. 2:1285, Nov. 22, 1958.

The author recommends that reports on diabetic children who leave school contain a note on the subject of employability. A brief discussion of types of employment both suitable and unsuitable for diabetics follows this suggestion. C.A.R.

Editorial. INSULIN ANTIBODIES. Brit. M. J. 2:1458-59, Dec. 13, 1958.

The author discusses the relation between insulin resistance and the presence of insulin antibodies in diabetic patients. Mention is made of recent studies of the fate of insulin labeled with radioactive iodine. A full interpretation of the phenomenon of insulin binding must await further work on the metabolism of insulin, both in normal subjects and in diabetics. C.A.R.

Friedlander, Ernest O.; and Bryant, David M., Jr. (Veterans Admin. Center, Togus, Me.): IDIOPATHIC INSULIN-RESISTANT DIABETES MELLITUS. Am. J. Med. *XXVI*:139-45, January 1959.

The authors present a case report of idiopathic insulin-resistant diabetes mellitus in a fifty-six-year old man whose resistance was associated with insulin allergy. ACTH gel reduced the daily insulin dosage of 750 U. to 125 U. per day. A temporary reduction in insulin requirement followed the administration of nitrogen mustard.

Intradermal injections of insulin and passive transfer tests confirmed the presence of insulin allergy. Insulin^{131} and starch-block electrophoresis demonstrated the insulin-binding antibodies to localize in the gamma globulin fraction. During a time when 98 per cent of insulin-I^{131} was bound to gamma globulin, the daily insulin dosage was 800 U. When 42 per cent was so attached, the daily insulin requirement was reduced to 270 U. daily.

During one interval, the administration of tolbutamide appeared to reduce the total insulin requirement but subsequently this could not be reversed by the administration of tolbutamide. Four and a half months of prednisolone therapy was followed by a reduction to 50 U. This continued up to the time of publication of the case report despite subsequent omission. R.W.S.

Galansino, Giorgio; D'Amico, Giuseppe; Kanameishi, Doris; and Foa, Piero P. (Dept. of Physiol. and Pharmacol., Chicago Med. Sch., Chicago 12, Ill.): MODE OF ACTION OF INSULIN, CARBUTAMIDE, AND TOLBUTAMIDE. Proc. Soc. Exper. Biol. & Med. 99:447-51, November 1958.

Varying doses of insulin were injected at different rates into the femoral and the portal veins of anesthetized dogs. No qualitative differences were noted between the two routes of administration. In all cases insulin caused a decrease in concentration of blood glucose and potassium and an increase in the concentration of pyruvate and lactate. Intravenous carbutamide and tolbutamide caused a decrease, not only in blood glucose and potassium, but also pyruvate and lactate. The authors suggest that the oral hypoglycemic agents may stimulate release of insulin from the pancreas and that when a minimum amount of insulin is available and glycogenolysis is not accelerated as in severe diabetes, these compounds may also inhibit glucose production by the liver or its utilization by the muscle. G.J.H.

Gepts, W. (From the Dept. of Pathology, University Hospital Brugmann and the Fondation Medicale Reine Elisabeth, Brussels, Belgium): THE HISTO-PATHOLOGICAL ALTERATIONS OF THE ISLETS OF LANGERHANS AND THEIR SIGNIFICANCE FOR THE PROBLEM OF THE PATHOGENESIS OF DIABETES MELLITUS IN MAN. Endokrinologie 36:185-211, July 1958.

The author describes a shift in alpha:beta cell ratio, with an increase of the alpha cells and a decrease of the beta cells as the most significant histopathologic finding in the pancreas of diabetic patients. The shift is far more significant in juvenile diabetics than in aged patients. This observation was made on the study of 142 pancreatic glands (forty-seven diabetic and ninety-five nondiabetic) which were examined within less than three hours post mortem and stained with Gomori's chrom-alum-hematoxylin method and with silver impregnation. With the Gomori technic the alpha cells of the nondiabetic islets varied between 10.5 per cent and 55.1 per cent of the total islet cells with an average of 31.2 per cent, those of the diabetic between 21.9 per cent and 72.4 per cent with an average of 47.4 per cent. The silver impregnation method gave almost consistently higher values in diabetics and nondiabetics, with less significant differences between both groups. That this shift in the alpha:beta cell ratio is due to an absolute decrease in the number of beta cells and not to an increase in the number of alpha cells was demonstrated by the quantitative correlation of pancreas-weight, islet-cell mass and alpha:beta cell ratio. The diabetic pancreas contains generally less islet cell tissue than the nondiabetic gland: This was shown in regard both to mass and to weight. The higher percentage of alpha cells in the diabetic pancreas thus is not manifested by an increase of islet cell mass or weight, and the total alpha-cell weight of the diabetic pancreas is not greater than that of the nondiabetic gland. The total beta-cell weight of the diabetic pancreas, on the other hand, is markedly smaller than that of the nondiabetic gland. Among the other histopathological diabetic islet-cell changes, the author discusses the hyalinosis as the most common alteration, the significance of which for the pathogenesis of the disease is, however, as yet unexplained. With regard to the hydropic degeneration, he states that it can be found also in glands of nondiabetic patients and that it cannot be considered as a causative factor of diabetes mellitus. In his series it was present in 40.7 per cent of the diabetic glands and in 5.3 per cent of the nondiabetic (German). M.G.G.

Gillman, Joseph; Gilbert, Christine; Epstein, Edward; and Allan, J. C. (Depts. of Physiol. and Anat. and Joint Nutrition Res. Unit and Surgery, University of Witwatersrand, Johannesburg, Union of South Africa): ENDOCRINE CONTROL OF BLOOD SUGAR, LIPAEMIA, AND KETONAEMIA IN DIABETIC BABOONS. Brit. M. J. 2:1260-63, Nov. 22, 1958.

Removal of the hypophysis from depancreatized baboons deprived of insulin did not alleviate the hyperglycemia but did prevent the excessive lipemia and abolish the ketonemia. It was demonstrated in depancreatized-hypophysectomized baboons that by the administration of cortisone the hyperlipemia, cholesterolemia, and phospholipemia could be raised to levels greater than those in the same animals before hypophysectomy, but unattended by ketonemia. A saline suspension of acetone-dried human anterior pituitary administered to depancreatized-hypophysectomized baboons scarcely affected the serum lipids but did produce a severe ketonemia and hyperglycemia. The complete diabetic picture was reproduced in depancreatized-hypophysectomized baboons by cortisone in conjunction with crude human anterior pituitary. From a consideration of the relevant data it was indicated that the individual differences in response to pancreatectomy were not only a function of the lack of insulin but of the intensity of participation of the adrenal cortex, of the thyroid gland, and of somatotrophic hormone in the metabolism of each baboon. C.A.R.

Gourley, D. R. H. (Dept. of Pharmacol., Univ. of Virginia Med. School, Charlottesville, Va.): RELATION BETWEEN STRUCTURE OF SULFONYLUREA COMPOUNDS AND THEIR EFFECT ON FROG MUSCLE METABOLISM. Proc. Soc. Exper. Biol. & Med. 99:69, October 1958.

The author presents evidence to demonstrate that the effects of sulfonylbutylurea derivatives on frog muscle metabolism as determined by measurements of oxygen consumption, depend upon the molecular groups substituted on the benzene ring para to the main chain of the molecule. Substitution of an amino group produces an inhibitory compound. The absence of any group or substitution of either a methoxy or a carboxy radical produced a compound with little or no effect. However, substitution of a methyl, ethyl, isopropyl, or tertiary butyl radical produces compounds that are increasingly effective in stimulating glycolysis and oxygen consumption. G.J.H.

Greenbaum, D.; and Bomford, Richard R. (Letter to Editor) (London Hospital, London, England): HYPOPHYSECTOMY FOR VASCULAR COMPLICATIONS OF DIABETES. Lancet 2:1288-89, Dec. 13, 1958.

The authors report an unimpressive result of hypophysectomy on a diabetic patient, in whom further progression of vascular complications continues following surgery. They make brief reference to other reports on the effects of this operation on the vascular complications of diabetes mellitus in which the reported results were similarly unimpressive. C.A.R.

Haunz, E. A. (Univ. of North Dakota, Grand Forks, N. D.): THE TOLBUTAMIDE DILEMMA. Journal-Lancet 78:287-89, July 1958.

In response to a questionnaire sent to ten eminent clinicians and investigators in the field of diabetes, the author received the following results:

Questionnaire

1. Do you feel that tolbutamide is being used too extensively and too indiscriminately by general physicians in your area? Yes, 6; no, 3; don't know, 1.

2. Have you encountered diabetic patients previously satisfactorily controlled on insulin who developed ketoacidosis on tolbutamide and who had to be re-established on insulin? Yes, 8; no, 2.

3. Have you seen any actual deaths from diabetic coma developing after tolbutamide therapy? No, 10.

4. Do you think practitioners in general often fail to heed established criteria for selection of diabetics for trial with tolbutamide? Yes, 7; no, 2; don't know, 1.

5. Do you believe tolbutamide is now an established and acceptable treatment in properly selected diabetics? Yes, 5; no, 5.

6. Have you sometimes found it very difficult to re-establish insulin treatment in so-called tolbutamide failures? Yes, 2; no, 8. W.R.K.

Henn, Mary J. (Univ. of Nebraska Coll. of Med., Omaha, Neb.): THE USE AND MISUSE OF THE SULFONYLUREAS IN DIABETES. Nebraska M. J. 43:495-98, November 1958.

The author relates when and how to use and when not to use oral hypoglycemic agents in treatment of diabetes. She makes clear the means of selecting cases that may be treated by oral medication and the proper management of patients to whom sulfonylureas are given. A case is cited to illustrate their misuse. W.R.K.

Hennes, Allen R.; and West, Kelly M. (Univ. of Oklahoma, School of Med. and Veterans Admin. Hosp., Oklahoma City, Okla.): THE PRESENT STATUS OF THE USE OF ORAL MEDICATIONS IN DIABETES MELLITUS: MECHANISM OF ACTION OF ORINASE. J. Oklahoma M. A. 51:648-54, November 1958.

Tolbutamide, when administered in addition to standard dietary therapy may be effective in controlling hyperglycemia in a selected group of diabetic patients. Such patients are usually over forty years of age. The dose most frequently employed is 0.5 gm. twice daily. The factors which influence the selection of patients for treatment with tolbutamide have been discussed, and the clinical use of this drug has been briefly described. B.F.K.

Hofstetter, J. R.; and Ramel, Cl. (Medical Policlinic, Univer. of Lausanne, Switzerland): THE USE OF SULFONYLUREA COMPOUNDS IN THE TREATMENT OF DIABETES MELLITUS. Schweiz. med. Wchnschr. 88:821-27, Aug. 23, 1958.

The action of the sulfonylurea compounds seems associated with persistence of some endogenous insulin production. The authors review the results of ambulatory treatment of 160 diabetics with carbutamide and tolbutamide. Only twenty-five patients failed to respond to the new drugs. Juvenile diabetics and cases of hemochromatosis are generally nonresponsive. No late failures were observed. It was not possible to reduce the insulin dosage by combining insulin therapy with sulfonylurea in patients refractory to the latter compound. This is in contrast to reports by other authors. Carbutamide has more side effects than tolbutamide. Occasionally this can be avoided by starting the treatment with very small doses, which are increased progressively. In a case of Addison's disease hypoglycemia with fatal result was observed. Vascular and infectious complications of diabetes appear not to be influenced by sulfonylurea compounds (German). M.G.G.

Johnston, Robert L. (Caylor-Nickel Clin. Hosp., Bluffton, Ind.): SUBSHOCK INSULIN THERAPY. M. Times 86:840-47, July 1958.

A discussion of insulin subshock therapy in the treatment of mental illness is presented. Indications, technic, and precaution in the use of insulin therapy are discussed. W.R.K.

Kalant, N.; Gomberg, C.; and Schucher, R. (Dept. of Investigative Medicine, McGill University; Research Lab., Jewish Gen. Hosp., Montreal, Canada): THE EFFECT OF INSULIN-BINDING ANTIBODIES ON INSULIN SENSITIVITY. Lancet 2: 614-17, Sept. 20, 1958.

1. Diabetic patients previously treated with insulin had less insulin response than diabetic patients who had not been previously treated. The insulin response was apparently not related to the degree of globulin-binding of insulin or to the degree of insulin retention in the circulation.

2. Repeated injection of insulin into normal rabbits led to the development of hemagglutinating antibodies to insulin; the removal of insulin from the circulation was altered, and binding of insulin to serum-globulin was demonstrable. There was no evidence of a decrease in hypoglycemic response to insulin or of presence of anti-insulin activity in serum.

3. Infusion, into normal rabbits, of serum obtained from insulin-treated diabetics led to the globulin-binding of insulin but did not alter the rate of insulin removal.

4. It is concluded that insulin-binding antibodies and hemagglutinating antibodies are not responsible for decreased responsiveness to insulin or for its slower removal from the circulation.

5. Moving boundary electrophoresis indicates that the insulin-binding antibodies are β-globulins. C.A.R.

Kinch, Robert A. H. (Dept. of Obs. & Gyn., Univ. of Western Ontario, London, Ont., Canada): FETAL HAZARDS IN THE DIABETIC PREGNANCY. Canad. M. A. J. 79:713-18, Nov. 1, 1958.

A detailed review of fifty-one consecutive viable pregnancies in thirty-four diabetic women from 1951 to 1957. The author advocates the same obstetric management of the mild "glucose tolerance" diabetic (but with later delivery) as for frank diabetic subjects. Patients are hospitalized at thirty-two weeks but delivery is postponed beyond thirty-four weeks. He emphasizes that fetal deaths are usually explainable on preventable medical or obstetric grounds except for prematurity. The commonest medical grounds are acidosis, toxemia and increasing hydramnios, and when discovered indicate prompt termination of pregnancy. Traumatic delivery is the avoidable obstetric hazard. He agrees with the prognostic classification of White including the adjustment of delivery time by class, but disagrees with the indication for hormonal therapy. S.B.B.

Meltzer, Lawrence E.; Bockman, Albert A.; and Berryman, George H. (Philadelphia Gen. Hosp., Philadelphia, Pa., and University of Illinois, Chicago, Ill.): A MEANS OF LOWERING ELEVATED BLOOD CHOLESTEROL LEVELS IN PATIENTS WITH PREVIOUS MYOCARDIAL INFARCTION. Am. J. M. Sc. 236:595-602, November 1958.

The authors report upon twenty-eight male patients aged thirty-six to fifty-three years who had sustained myocardial infarctions and who exhibited persistently elevated serum cholesterol levels when followed upon a relatively constant dietary fat intake over a period of at least six months. The patients were subdivided into three groups based on the daily fat intake. Group I consisted of ten patients on less than 50 gm. daily; group II of eight patients on 50-100 gm. daily; and group III, ten patients on 100-150 gm. daily. During the test period all subjects were continued on their previous fat intake and previous type of work activity but the diet was supplemented with four tablespoons daily of a safflower oil emulsion, whose natural plant sterol content (0.41 per cent) was fortified artificially to 2 per cent, the maximum compatible with solubility and palatability. One tablespoonful of this emulsion provided 300 mg. plant sterols, 1.0 mg. pyridoxine and 9.8 gm. of safflower oil which contained 20 mg. vitamin E. This formula is referred to hereafter as "S-S-P" (Safflower oil-Sitosterol-Pyridoxine). All subjects showed a decrease in baseline blood cholesterol at the end of a three-month treatment period. This decrease was maintained or further lowered during a subsequent three-month period during which the over-all reduction in blood cholesterol at the end of six months was significant for all patients in group I and for seven of eight and seven of ten in groups II and III respectively. The greatest decline in blood cholesterol was noted with low and intermediary dietary fat intakes when weight gain was slight or absent. In no case was there an elevation over the original baseline value even in the presence of considerable weight gain. L.S.S.

Murray, Ian; Riddell, M. J.; and Wang, Ian. (Victoria Infirmary, Glasgow, Scotland): CHLORPROPAMIDE. Lancet 2: 749, Oct. 4, 1958.

The authors comment briefly on the use of chlorpropamide in diabetic patients and emphasize that this drug, as well as other hypoglycemic agents, is much less effective in the presence of infection or other illness in which increased insulin requirement is usually present. C.A.R.

Nath, M. D.; and Bhattathiry, E. P. M. (Univ. Dept. of Biochem., Nagpur, India): CURE OF ALLOXAN DIABETES BY COMBINED THERAPY WITH GLUCOSE-CYCLO-ACETOACETATE AND ESTROGEN. Metabolism *VIII*:120-25, March 1959.

The authors demonstrate amelioration of alloxan diabetes in rabbits by the daily intramuscular injection of glucose-cycloacetoacetate as the sodium salt and estrogen (estradiol monobenzoate). When glucose-cycle-acetoacetate was hydrolyzed prior to administration, the effect was more pronounced. The treated animals were found to be normal in all respects. Neither compound affected the cure when given alone. The authors postulate that the curing effect of this combination may be explained by assuming a synergism between glucose-cycloacetoacetate and estrogen, the former spare glutathione and the other by activating hexokinase. R.W.S.

Odell, William D.; Tanner, Donald C.; Steiner, Donald F.; and Williams, Robert H. (University of Washington, School of Medicine, Seattle, Wash.): PHENETHYL-, AMYL-, AND ISOAMYLBIGUANIDE IN THE TREATMENT OF DIABETES MELLITUS. A. M. A. Arch. Int. Med. 102:520-26, October 1958.

A preliminary report is presented dealing with the oral treatment of diabetic patients with amyl-, isoamyl-, and phenethylbiguanide. Fifty-two per cent of the patients treated had a "good" response, which was defined as a decrease of the fasting blood sugar by 30 per cent or more, with at least a 50 per cent decrease in glycosuria or a decrease in insulin requirements 30 per cent or more.

Treatment was hindered markedly by the high incidence of side effects, and these were of two types: (a) Early side effects consisting of anorexia, nausea, vomiting, or very rarely diarrhea usually occurring in the first week of therapy, were seen to some extent, though usually in mild form, in 60 per cent of the patients treated. (b) Late side effects occurred after four

to sixteen weeks of apparently successful therapy in ten out of twenty-eight patients treated for one month or longer. These consisted of weight loss, weakness, lethargy, and malaise. All side effects, early and late, generally disappeared from one to three days after the biguanide therapy was discontinued and in all cases were gone within one week. No permanent effects of any kind have been observed, in spite of careful search.

There was no correlation between age of the patient, duration of diabetes and/or insulin therapy, and response to biguanide therapy, but there was an inverse correlation between the dose of insulin necessary to control the diabetes and the percentage of patients responding to biguanide therapy.

Diabetic acidosis occurred in two patients while on biguanide therapy. Their blood sugar level fell to normal or below on remarkably small doses of insulin. In some cases the severely labile diabetic patient, who could not be controlled on insulin, was well controlled on insulin plus a biguanide. Although in many diabetic patients biguanide therapy produced excellent results, its use was severely hampered by the high incidence of side effects. Final evaluation must await a more thorough elucidation of its biochemical effects, for it is possible that with such knowledge side effects might be eliminated while the hypoglycemic effect is preserved. B.F.K.

Randle, P. J.; and Taylor, K. W. (Dept. of Biochemistry, University of Cambridge, England): INSULIN IN PROTEIN FRACTIONS OF SERUM FROM HEALTHY PEOPLE AND FROM INSULIN-TREATED DIABETICS. Lancet 2:996-97, Nov. 8, 1958.

The authors refer to their previous investigations of the insulin fractions in serum from healthy subjects and diabetic patients. The diabetic group included both insulin-treated patients and those treated with diet alone. The present observations, in the form of a preliminary report, appear to show that the patterns of distribution of insulin in protein fractions of serum from healthy people and insulin-treated diabetics are similar. A full account of these investigations will be submitted for publication elsewhere. C.A.R.

Reid, James (Clinical Chemotherapeutic Res. Unit, Medical Research Council, Western Infirmary, Glasgow, Scotland): DINITROPHENOL AND DIABETES MELLITUS: A COMPARISON WITH SALICYLATE. Brit. M. J. 2:724-27, Sept. 20, 1958.

Salicylate and 2:4-dinitrophenol (DNP) inhibit oxidative phosphorylation. Salicylate also controls moderately severe diabetes mellitus. The effect of DNP in the disease has been investigated. Increased oxygen consumption from DNP of the same order as is required with aspirin to control moderately severe diabetes lowered fasting blood sugar and glycosuria of six elderly diabetics. The reduction was, however, less than that achieved with aspirin, and would have no practical value in treatment. This difference between DNP and salicylate in diabetes makes it difficult to account for the action of both substances on the basis of inhibition of oxidative phosphorylation, at least, without further specifications. C.A.R.

Rubin, Alan; and Babbott, David (School of Med. and Hosp., University of Pennsylvania, Philadelphia, Pa.): IMPOTENCE AND DIABETES MELLITUS. J.A.M.A. *168*:498-500, Oct. 4, 1958.

One hundred ninety-eight men attending outpatient clinics for the treatment of diabetes mellitus were studied in relation to frequency of coitus, erectile and ejaculatory impotence, premature ejaculation, libido, morning erections, contraception and venereal disease. The incidence of erectile impotence at all ages was found to be two to five times higher than that reported by Kinsey and associates. Twenty-five per cent of men between thirty and thirty-four years of age who had diabetes suffered from impotence. The incidence gradually increased with age to 53.6 per cent for men fifty to fifty-four years of age. Thirty per cent of those who became impotent did so within one year after the clinical recognition of their diabetes. Sixty per cent became impotent within five years of the onset of their disease.

There was no apparent relationship between the age at onset of the diabetes, its duration or severity, or the presence of vascular, neurologic, or other complications and the incidence of impotence. Libido usually persisted for some time after the appearance of impotence, even though all erections, including morning erections, often were absent. Prior to the clinical onset of the diabetes there was no unusual incident of impotence. It is suggested that men complaining of premature impotence be investigated for diabetes mellitus. B.F.K.

Rubini, Milton E.; and Seligson, David (Dept. of Metabolism, Walter Reed Army Inst. of Res., Walter Reed Army Med. Center, Wash., D.C.): THE ACUTE REDUCTION IN PLASMA AMINO ACIDS BY CARBOHYDRATE INFUSION IN DIABETES AND LIVER DISEASE. Am. J. Clin. Nutrition 6:365-75, July-August 1958.

The authors test the hypothesis that the acute decrease in the plasma amino acid pool following exercise, insulin, and glucose feeding in the normal animal and man reflects either a decrease in protein breakdown or an increase in protein synthesis. Insulin or exercise, superimposed upon previous carbohydrate infusion (glucose or fructose) was not found to decrease the plasma amino acids further. Diabetic patients, selected as likely to be insulin deficient, as well as normal dogs given large amounts of glucose to produce hyperglycemia demonstrated a normal plasma amino acid fall. The authors suggest that the fall in plasma amino acids is related to the increased rate of carbohydrate utilization and is largely independent of the level of blood sugar or the action of insulin. Choline-deficient dogs with fatty liver, and about 50 per cent of a diverse group of patients with liver disease, responded abnormally. These data indicate that intact hepatocellular function is necessary for the reduction of plasma amino acids following carbohydrate infusion, and suggest that the liver is a major site of removal of amino acids from the plasma under these circumstances. R.L.J.

Shields, Charles E. (Tripler Army Hosp., APO 438, San Francisco, Calif.): DIABETIC ACIDOSIS WITH TRANSIENT RENAL INSUFFICIENCY. U. S. Armed Forces M. J. 9:1351-58, September 1958.

A case of diabetic ketosis occurring in a thirty-two-year-old woman without known antecedent renal disease is presented. During therapy a thirty-one-hour period of oliguria occurred. Diuresis and recovery followed the administration of potassium chloride. It has been emphasized that transient renal insufficiency may have had its inception secondary to the induced hypernatremia and concomitant hypokalemia. Various pathophysiologic and experimental data have been submitted to support this thesis. C.A.R.

Siperstein, Marvin D.; and Fagan, Violet M. (Univ. of Texas, Southwestern Med. School, Dept. of Internal Med.; Dallas, Tex.): STUDIES ON THE RELATIONSHIP BETWEEN GLUCOSE OXIDATION AND INTERMEDIARY METABOLISM. I. THE IN-

FLUENCE OF GLYCOLYSIS ON THE SYNTHESIS OF CHOLESTEROL AND FATTY ACID IN NORMAL LIVER. J. Clin. Invest. 37:1185-95, August 1958.

The processes of lipogenesis and cholesterolgenesis from C^{14}-labeled acetate were studied in liver homogenates of the normal rat. Stimulation of glycolysis by the Embden-Meyerhof (EM) pathway had a variable effect, but that by the hexose-monophosphate shunt (HMP) increased both processes markedly. Stimulation of both types of glycolysis caused a still greater increase in the rate of lipogenesis, but a decrease in cholesterolgenesis. TPNH generated by another system (isocitrate and TPN) could effect a similar increase. It was concluded that the cofactor TPNH produced as a product of HMP glycolysis was the limiting factor in normal hepatic lipogenesis and cholesterolgenesis. S.B.B.

Szucs, M. M.; Eusebi, A. J.; and Petraglia, Samuel (St. Elizabeth's Hosp., Youngstown, Ohio): THE ARYLSULFONYLUREAS: TWELVE-MONTH CLINICAL STUDY IN 814 PATIENTS WITH DIABETES MELLITUS. Am. Pract. & Digest Treat. 9:1415-18, September 1958.

A review of experiences of more than twelve months with successful tolbutamide therapy of 664 clinic adult diabetic patients. There were in addition 150 patients with primary failure of therapy, of whom a third were juvenile diabetics. The usual dosage range was 1.0 to 1.5 gm. per day. There was no close correlation of success in therapy with either duration of therapy or age (in the maturity onset diabetics). S.B.B.

Tolstoi, Edward (Dept. of Medicine, The New York Hosp. and Cornell University Med. College, New York, N. Y.): SUBSTANCES USED IN TREATMENT OF DIABETES MELLITUS. Am. J. M. Sc. 236:625-33, November 1958.

The author comprehensively reviews the two classes of substances currently available for the treatment of diabetes mellitus —the insulins and the sulfonylureas. While not all clinicians would agree in toto with the view that the intermediary insulins, NPH, Globin and Lente, are practically interchangeable unit for unit and that to treat almost all of the facets of diabetes, the healthy and the sick, one needs only two types of insulin: the fast-acting regular crystalline or Semilente insulin for all emergencies and an intermediary insulin for the ambulatory healthy diabetic; yet few would dissent from the opinion that emergency situations such as ketosis, coma, surgical patients and infections must be treated with the most rapidly acting insulin, namely, crystalline or regular. This type alone can be administered intravenously.

With insulin, all diabetics, young or old, mild or severe. can be successfully treated, the only hazard being the possibility of hypoglycemic reactions and the only contraindication the rare case of generalized allergy to the hormone. Again, while not all authorities would accept the dictum that glycosuria is desirable at all times in patients taking slow-acting insulins in order to avoid hypoglycemic coma, none would differ with the recommendation that the patient allergic to insulin should be desensitized before an emergency arose and that the patient resistant to insulin should be vigorously treated to eliminate ketosis regardless of dosage or the period of its administration until the resistant state had run its self-limited course and a more average insulin unitage could be resumed.

By contrast to the insulins, the sulfonylurea drugs have a limited area of usefulness in the field of diabetic therapy and are applicable only to a selected group of patients usually designated as middle-aged, nonobese and requiring 20 to 40 units of insulin daily to be controlled with nearly normal blood sugars and no or minimal glycosuria. While the majority of reports claim success in the clinical trial with tolbutamide for this segment of the diabetic population, the author repeats Goldner's and Weisenfeld's warning "there is a difference between successful clinical trials and clinical indications for a drug. The purpose of clinical trials is to establish the widest possible parameter of effectiveness. The clinical indications, on the other hand, are those conditions where a particular drug is needed or specifically effective and advantageous." The clinical indications for tolbutamide are still to be defined. Definition must await discovery of how the sulfonylureas exert their hypoglycemic action and clarification of the exact relationship between lowering of the blood sugar and the treatment of diabetes. Certainly tolbutamide is not indicated for the diabetic who can be managed readily by dietary means alone, who rapidly develops ketosis, whose response is only transitory, and who exhibits a side effect such as leukopenia or a dermatosis. There is general agreement upon a maintenance dose of tolbutamide of 0.5 to 1.5 gm. daily after larger initial priming doses and with the view that none of the currently employed preliminary tests predicts with certainty the successful candidate for the drug. At this time one must be cautious when using the sulfonylureas. L.S.S.

Vallance-Owen, J.; Dennes, Elizabeth; and Campbell, P. N. (Postgraduate Med. School of London, W. 12; Courtauld Institute, Middlesex Hosp., London, W. 1, England): THE NATURE OF THE INSULIN-ANTAGONIST ASSOCIATED WITH PLASMA-ALBUMIN. Lancet 2:696, Sept. 27, 1958.

The authors have recently shown that the inhibitor of insulin in the plasma of diabetic patients is associated with the albumin fraction. They have also shown that albumin from normal subjects has an inhibitory action. They now present evidence that the inhibitory activity in such plasma is due to a substance associated with the albumin. Three hypophysectomized patients were studied. These patients were cortisone-dependent. The albumin from the plasma of these patients was tested for insulin antagonism, but none could be demonstrated. Albumin from the plasma of the other diabetic patients was prepared by utilizing a cellulose column. When the activity of this protein, which was electrophoretically identical with albumin, was tested, the original activity against insulin was found to have been removed by passage through the column. A similar result was obtained when normal albumin was passed through a column under similar conditions. Both of these lines of evidence suggest that the activity of the albumin fraction from normal subjects and diabetic patients against insulin is not due to albumin per se. The results of hypophysectomy indicate that the antagonistic activity is related, possibly indirectly, to the pituitary gland. C.A.R.

Wells, Ronald (Dept. of Med., Gen. Hosp., Singapore, Indonesia): REMITTENT INSULIN-INSENSITIVE DIABETES. Brit. M. J. 2:1328-31, Nov. 29, 1958.

The author discusses a diabetic syndrome in eight patients characterized by marked insulin insensitivity and periods of complete or partial remission. He does not distinguish mild diabetes well controlled by diet from a true diabetic remission. C.A.R.

ORGANIZATION SECTION

OFFICERS AND MEMBERS OF COUNCIL, AMERICAN DIABETES ASSOCIATION, 1958-1959

HONORARY PRESIDENT, ELLIOTT P. JOSLIN, M.D., *Boston*

PRESIDENT
ALEXANDER MARBLE, M.D., *Boston*

FIRST VICE PRESIDENT
FRANCIS D. W. LUKENS, M.D., *Philadelphia*

SECOND VICE PRESIDENT
FRANKLIN B. PECK, SR., M.D., *Indianapolis*

SECRETARY
E. PAUL SHERIDAN, M.D., *Denver*

TREASURER
THOMAS P. SHARKEY, M.D., *Dayton*

EXECUTIVE DIRECTOR
J. RICHARD CONNELLY, *New York*

MEMBERS OF COUNCIL

TERM EXPIRING 1959

LOUIS K. ALPERT, M.D., *Washington, D.C.*
W. WALLACE DYER, M.D., *Philadelphia*
EDWIN W. GATES, M.D., *Niagara Falls*
HARVEY C. KNOWLES, JR., M.D., *Cincinnati*
ARNOLD LAZAROW, M.D., *Minneapolis*
LAURENTIUS O. UNDERDAHL, M.D., *Rochester, Minn.*

TERM EXPIRING 1960

CHARLES H. BEST, M.D., *Toronto*
THADDEUS S. DANOWSKI, M.D., *Pittsburgh*
EDMOND K. DOAK, M.D., *Houston*
GARFIELD G. DUNCAN, M.D., *Philadelphia*
HENRY E. MARKS, M.D., *New York*
LEON S. SMELO, M.D., *Birmingham*

TERM EXPIRING 1961

JOSEPH T. BEARDWOOD, JR., M.D., *Philadelphia*
ARTHUR R. COLWELL, SR., M.D., *Chicago*
JOSEPH H. CRAMPTON, M.D., *Seattle*
LAURANCE W. KINSELL, M.D., *Oakland*
RACHMIEL LEVINE, M.D., *Chicago*
KELLY M. WEST, M.D., *Oklahoma City*

PAST PRESIDENTS

HENRY T. RICKETTS, M.D., *Chicago;*
FREDERICK W. WILLIAMS, M.D., *New York;* JOHN A. REED, M.D., *Washington, D.C.*

EX OFFICIO

CHRISTOPHER J. MCLOUGHLIN, M.D., *Atlanta,* Chairman Board of State Governors
HENRY E. OPPENHEIMER, M.D., *St. Louis,* Chairman Assembly of Delegates

19th Annual Meeting
American Diabetes Association
June 6-7, Chalfonte-Haddon Hall, Atlantic City, New Jersey

"The Adrenal and Diabetes: Some Interactions and Interrelations" is the title of the Banting Memorial Lecture of the American Diabetes Association which will be delivered by George W. Thorn, M.D., at the Nineteenth Annual Meeting. The Lecture will be given from 10:55 to 11:55 during the Scientific Session Saturday morning, June 6. Dr. Thorn is Hersey Professor of the Theory and Practice of Physic, Harvard Medical School; and Physician-in-Chief, Peter Bent Brigham Hospital, Boston. He will be presented with the Banting Medal at the Annual Banquet of the Association to be held that evening. The Banting Medal also will be awarded to E. T. Bell, M.D., Emeritus Professor of Pathology, University of Minnesota, Minneapolis, "for distinguished service in the interest of doctor and patient."

Alexander Marble, M.D., will deliver the Address of the President. The invocation will be offered by Deaconess Maude Behrman of Atlantic City, and the principal speaker will be Boland Hughes, M.D., Professor of Clinical Urology, University of Pennsylvania School of Medicine, Philadelphia. His address is entitled "The Contemporary Scene in the U.S.S.R."

The 1959 Lilly Award also will be presented at the Banquet to Marvin D. Siperstein, M.D., Ph.D., Associate Professor of Internal Medicine, University of Texas Southwestern Medical School, Dallas. The award, supported annually by Eli Lilly and Company, consists of $1,000 and a medal. Its purpose is to recognize demonstrated research in the field of diabetes, taking into consideration independence of thought and originality.

Approximately thirty committees and boards are scheduled to meet, and notices of time and place have gone forward to members of these groups. In addition, a complete list of committee and other meetings appears in the printed program.

Although a joint meeting will not be held with The Endocrine Society, arrangements have been made so that each organization will recognize the registration badges of the other. Endocrine Society members, therefore, may attend the Scientific Sessions of the American Diabetes Association and ADA members may attend, without charge, the session on diabetes of The Endocrine Society. The latter will be held at the Chalfonte at 2:00 p.m., Saturday, June 6.

Copies of the ADA program, containing abstracts of papers to be presented as well as those included by title, will be available at the registration desk at $1.00 each. A copy at no cost is being sent to all members in advance of the meeting; however, the aforementioned charge will be made for copies secured at the time of registration.

The Annual Business Meeting will be held in the Vernon Room Sunday, June 7, from 12:00 to 12:30 p.m., a change in time from that in previous years. The Annual Banquet will be held in the Vernon Room on Saturday, June 6, at 7:30 p.m., and will be preceded by a Social Hour at 6:30 p.m. in the English Lounge.

Papers to be presented at the Scientific Sessions and those included in the program by title are listed here for the information of readers of the Journal.

SCIENTIFIC SESSIONS

Saturday, June 6, 9:00 a.m.

ALEXANDER MARBLE, Presiding

1. **Studies on the Diabetogenic Action and the Site of Origin of Glucagon**
BRUNO W. VOLK (by invitation), and SYDNEY S. LAZARUS, Isaac Albert Research Institute, Jewish Chronic Disease Hospital, Brooklyn, and Albert Einstein College of Medicine, Bronx, N.Y.

2. **Significance of Circulating Lipids in the Management of Diabetes Mellitus**
DAVID ADLERSBERG, LISBETH EISLER (by invitation), IWAO FUKUI (by invitation), and PEH-PING HO (by invitation), The Mount Sinai Hospital, New York, N.Y.

3. **Radioactive Fat Tolerance Tests in Diabetes**
DONALD BERKOWITZ (by invitation), DAVID M. SKLAROFF (by invitation), and WILLIAM LIKOFF (by invitation), Albert Einstein Medical Center, Northern Division, Hahnemann Medical College and the Sidney Hillman Medical Center, Philadelphia

4. **The Significance of Nonatheromatous Intramural Vascular Lesions of the Heart in Diabetes Mellitus**
SIDNEY GOLDENBERG, MORRIS ALEX (by invitation), RAM A. JOSHI (by invitation), and HERMAN T. BLUMENTHAL (by invitation), The Jewish Hospital and Washington University and St. Louis University Schools of Medicine, St. Louis

5. **Plasma Insulin Activity after Glucose: An Index of Insulogenic Reserve in Normal and Diabetic Man**
HOLBROOKE S. SELTZER and WALTER L. SMITH (by invitation), University of Texas Southwestern Medical School and the Veterans Administration Hospital, Dallas, Tex.

BANTING MEMORIAL LECTURE

The Adrenal and Diabetes: Some Interactions and Interrelations
GEORGE W. THORN, Hersey Professor of the Theory and Practice of Physic, Harvard Medical School, Physician-in-Chief, Peter Bent Brigham Hospital, Boston, Mass.

6. **The Clinical Applicability of the Lente Insulin Triad**
FRED W. WHITEHOUSE, W. L. LOWRIE, W. E. REDFERN and J. B. BRYAN, Henry Ford Hospital, Detroit, Mich.

7. **Diabetic Diarrhea with Malabsorption Syndrome**
MAX ELLENBERG and JOHN J. BOOKMAN, The Mount Sinai Hospital, New York, N.Y.

8. **Diabetic Acidosis: A Six-year Review with an Evaluation of the Plasma Acetone Method**
AGUSTIN M. de ANDINO, Jr., LILLIAN HADDOCK-SUAREZ (by invitation), and RAUL COSTAS (by invitation), University of Puerto Rico School of Medicine, San Juan, P.R.

9. **The Fate of the Second Leg in the Diabetic Amputee**
MARTIN G. GOLDNER, Jewish Chronic Disease Hospital, Brooklyn

Sunday, June 7, 9:00 a.m.

ALEXANDER MARBLE, Presiding

10. **Intraportal Insulin and Tolbutamide Studies in Man and Portacaval Shunt Dogs**
THOMAS F. SHELLEY (by invitation), JOHN CINCOTTI (by invitation), EDWARD MARGULIES (by invitation), and THOMAS F. FRAWLEY, Albany Medical College, Albany, N.Y.

11. **Changes in Blood Sugar and Serum Radioactivity Produced by the Intravenous and Intraportal Administration of I^{131}-labeled Insulin in Dogs**
F. I. R. MARTIN (by invitation), JACK R. LEONARDS and MAX MILLER, Western Reserve University School of Medicine, Cleveland

12. **The Effect of Slow Infusion of Glucagon-free Insulin on the Hepatic Output of Glucose in Dogs with Portacaval Shunts**
LEONARD L. MADISON, BURTON COMBES (by invitation), and WILLIAM STRICKLAND (by invitation), University of Texas Southwestern Medical School, Dallas, Tex.

13. **Demonstration of Insulin-binding in Peripheral Tissues of Man and Its Relationship to Insulin Action**
ROGER H. UNGER, LEONARD L. MADISON, MARY S. McCALL (by invitation), ANN EISENTRAUT (by invitation), and SUSAN KELLER (by invitation), Veterans Administration Hospital and University of Texas Southwestern Medical School, Dallas

14. **On the Mechanism of Insulin Action**
R. C. de BODO, R. STEELE (by invitation), N. ALTSZULER (by invitation), A. DUNN (by invitation), D. T. ARMSTRONG (by invitation), and J. S. BISHOP (by invitation), New York University College of Medicine, New York, N.Y., and Brookhaven National Laboratory, Upton, N.Y.

15. **Inhibition of Insulin Degradation by Normal Plasma: Effects on in Vitro Bio-assay Systems**
EUGENE U. PIAZZA (by invitation), CHARLES J. GOODNER (by invitation), and NORBERT FREINKEL, The Boston City Hospital and Harvard Medical School, Boston, Mass.

16. **Clinical Studies of Insulin I^{131} Partitions in Diabetic Patients**
THADDEUS E. PROUT and ROBERT B. KATIMS, The Johns Hopkins University School of Medicine and The Johns Hopkins Hospital, Baltimore, Md.

17. **The Lack of Specificity of the Rat Epididymal Fat Pad as a Method for Insulin Assay of Blood**
JACK R. LEONARDS, Western Reserve University School of Medicine, Cleveland, Ohio

18. **Studies on the Lipogenic Defect of Diabetes**
MARVIN D. SIPERSTEIN, University of Texas Southwestern Medical School, Dallas, Tex.

Sunday, June 7, 2:00 p.m.

FRANCIS D. W. LUKENS, Presiding

19. **Formation of Oligosaccharides and Glycogen from Glucose and from Galactose by Rat Diaphragm**
I. ARTHUR MIRSKY, GLADYS PERISUTTI (by invitation), and NEIL C. DAVIS (by invitation), University of Pittsburgh School of Medicine, Pittsburgh, Pa.

20. **Hyperglycemic and Hypoglycemic Effects of Human Growth Hormone in Man**
ELEANOR McGARRY and JOHN C. BECK, McGill University Clinic, Montreal, Canada

21. **Comparative Pharmacology of Tolbutamide, Chlorpropamide, Metahexamide and Carbutamide in Man**
KELLY M. WEST, University of Oklahoma School of Medicine, Oklahoma City, Okla.

22. **Tolbutamide-induced Amelioration of Diabetes of Young People with Restoration of Normal Carbohydrate Tolerance in Some**
STEFAN S. FAJANS and JEROME W. CONN, University of Michigan Medical School, Ann Arbor, Mich.

23. **Metahexamide and Methoxybenzenesulfonylaminoisobutylthiadiazole in the Oral Therapy of Diabetes**
RICHARD H. POLLEN (by invitation), ROBERT H. WILLIAMS, ROBERT H. BARNES, WILLIAM KELLY (by invitation), and DONALD C. TANNER, University of Washington School of Medicine, Seattle, Wash.

24. **A Clinico-pathologic Study of Oral Hypoglycemic Agents**
J. M. B. BLOODWORTH, Jr., and GEORGE J. HAMWI, Ohio State University College of Medicine, Columbus, Ohio

PANEL DISCUSSION

Oral Hypoglycemic Agents

GARFIELD G. DUNCAN, Moderator, University of Pennsylvania School of Medicine, Pennsylvania Hospital and Benjamin Franklin Clinic, Philadelphia, Pa.

WILLIAM S. COLLENS, Maimonides Hospital and State University of New York, Downstate Medical Center, Brooklyn, N.Y.

LEO P. KRALL, Joslin Clinic, Boston, Mass.

I. ARTHUR MIRSKY, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

ROBERT H. WILLIAMS, University of Washington School of Medicine, Seattle, Wash.

BY TITLE

Incidence of Glaucoma in Patients with Diabetes Mellitus
JOHN ARMSTRONG (by invitation), LOUIS GIRARD (by invitation), and HAROLD L. DOBSON, Baylor University College of Medicine and Jefferson Davis Hospital, Houston, Tex.

Oral Hypoglycemic Agents in the Treatment of Diabetes Mellitus
WILLIAM S. COLLENS and MORRIS M. BANOWITCH, Maimonides Hospital and the State University of New York, Downstate Medical Center, Brooklyn, N.Y.

The Full Diabetic Syndrome
HAROLD L. DOBSON, HOOSHANG GUILAK (by invitation), JOHN SCOGIN (by invitation), and JAMES A. GREENE, Baylor University College of Medicine and Jefferson Davis Hospital, Houston, Tex.

Kinetics of Glucose Utilization: II. The Influence of Age and Diabetes
HAROLD ELRICK and C. J. HLAD, Jr. (by invitation), Veterans Administration Hospital and University of Colorado School of Medicine, Denver, Colo.

The Effect of Endogenous Insulin Secretion upon the Magnitude of Hepatic Binding of Labeled Insulin During a Single Transhepatic Circulation in Human Subjects
NORMAN KAPLAN (by invitation), and LEONARD L. MADISON, University of Texas Southwestern Medical School, Dallas

Comparative Clinical and Laboratory Studies of Two Potent Oral Hypoglycemic Agents
THOMAS H. LAMBERT, SOLON PALMER, Jr. (by invitation), and ROY B. JOHNSON, Jr. (by invitation), Scripps Clinic and Research Foundation, La Jolla, Calif.

Sulfa-urea Treatment in Juvenile Diabetes
YNGVE LARSSON (by invitation), Kronprinsessan Lovisa's Children's Hospital, Caroline Institute, Stockholm, Sweden

Probable Variations in Adrenal Cortical Responsiveness among Diabetics Based on Leukocyte Changes Following Glucose Administration
MALCOLM S. MACKENZIE and MARJORIE B. SEASTONE (by invitation), Western Reserve University School of Medicine, Cleveland, Ohio

Diabetes Mellitus and the Convulsive State: Possible Genetic and Clinical Relationship
FRANK S. PERKIN and A. J. DERBYSHIRE (by invitation), Wayne State University College of Medicine, The Michigan Epilepsy Center and Harper Hospital, Detroit, Mich.

Degenerative Lesions in Dogs with Experimental Diabetes
HENRY T. RICKETTS, University of Chicago, Chicago, Ill.

Correlation of Glucose Levels in Tears and Saliva with the Blood Sugar Level
BARRY M. SEGAL (by invitation), and ARTHUR H. DUBE, State University of New York, Upstate Medical Center, Syracuse, N.Y.

Chlorpropamide Therapy in Outpatient Diabetic Patients
ASA J. SMITH and ARTHUR H. DUBE, State University of New York Medical Center, Syracuse, N.Y.

Experiences with Chlorpropamide in Fifty Clinic Patients—Comparative Study with Insulin and Tolbutamide
ROBERT C. STEWART (by invitation), EUGENE U. PIAZZA (by invitation), and DAVID HURWITZ, The Boston City Hospital, Boston, Mass.

The Frequency of Diabetes Mellitus in Cases of Anemia Perniciosa
ARNE SUNDBERG (by invitation), and ALBERT GRONBERG, Centrallasarettet, Vanersborg, Sweden

The Use of Intravenous Tolbutamide in Designing Therapy for Patients with Diabetes
C. ROBERT TITTLE, Jr., JOSEPH T. BEARDWOOD, Jr., and D. E. PALABIYIKOGLU (by invitation), Graduate Hospital, University of Pennsylvania, and Abington Memorial Hospital, Philadelphia, Pa.

The Significance of "Normal" and "Abnormal" Oral Glucose Tolerance Curves as Determined by Five-to-ten-year Follow-up in 200 Subjects
ROGER H. UNGER, LEONARD L. MADISON and ROSA M. ADAIR (by invitation), Veterans Administration Hospital, University of Texas Southwestern Medical School, and the City Health Department, Dallas, Tex.

POSTGRADUATE COURSE SERIES IN DIABETES AND BASIC METABOLIC PROBLEMS

The EIGHTH POSTGRADUATE COURSE will be held by the American Diabetes Association on Jan. 20, 21 and 22, 1960, in Los Angeles, California. The tentative program will include these major topics: "Fundamentals of Diabetes," "Diagnostic and Therapeutic Considerations," "Clinical Sessions in Diabetes or Diabetes in Review," "Vascular Complications of Diabetes," and "Diabetes on the Research Frontier."

Thaddeus S. Danowski, M.D., Chairman of the ADA Committee on Professional Education, will be Course Director, and Roy F. Perkins, M.D., the Association's Governor for Southern California, chairman of the local committee.

Registration fees are $40 for Association members and $75 for nonmembers. All inquiries pertaining to the Course should be directed to the national office, 1 East 45th St., New York 17, N.Y.

The SEVENTH POSTGRADUATE COURSE offered by the American Diabetes Association was held in St. Louis, Missouri, Jan. 21, 22 and 23, 1959. Developed by the ADA Committee on Professional Education, the Course had as its Director, William H. Olmsted, M.D., of St. Louis, and Henry E. Oppenheimer, M.D., St. Louis, as Co-Director. Round-table conferences, a new feature in our Postgraduate Series, were held on January 22 at the close of the afternoon session. Registrants were divided into groups of sixteen or less, so that questions could be addressed more easily to individual discussion leaders, who included: Frank N. Allan, M.D.; Charles H. Best, M.D.; Arthur R. Colwell, Sr., M.D.; T. S. Danowski, M.D.; Garfield G. Duncan, M.D.; Harvey C. Knowles, Jr., M.D.; Rachmiel Levine, M.D.; Francis D. W. Lukens, M.D.; Alexander Marble, M.D.; Max Miller, M.D.; Franklin B. Peck, Sr., M.D.; Henry T. Ricketts, M.D.; William C. Stadie, M.D.; and Frederick W. Williams, M.D.

A registration breakdown for the Seventh Postgraduate Course, and a comparison with the six previous Courses, follow:

REGISTRANTS	1959 St. Louis	1958 Atlanta	1957 Columbus	1956 Dallas	1955 Philadelphia	1954 Rochester	1953 Toronto
Members	55	78	83	82	135	83	93
Membership applications pending	21	47	55	52	23	24	23
Nonmembers	31	53	57	47	18	17	61
TOTAL	107	178	195	181	176	124	177
OTHERS ATTENDING							
Part-time registrants	2	1	1	—	2	—	—
*Graduate Students	—	—	—	—	1	†	†
*Fellows	7	1	—	2	3	†	†
*Residents	10	14	16	10	10	†	†
*Interns	8	4	8	4	5	†	†
*Medical Students	23	2	13	61	11	†	†
*Dietitians, Dietetic Interns	2	—	16	—	—	—	—
*Nutritionists	—	—	2	—	—	—	—
*Nurses	—	—	7	—	—	—	—
Councilors and Faculty	41	40	34	48	41	35	35
Guests	7	6	12	4	6	—	2
TOTAL	100	68	109	129	79	35	37
TOTAL OF ALL CATEGORIES	207	246	304	310	255	159	214
ADDITIONAL DATA							
Cancellations	8	19	12	12	15	25	5
Applications rejected due to limited facilities	—	—	—	—	—	47	92
Attendance at Banquet	173	245	200	324	246	208	236

*From metropolitan area of Course location.

†Graduate Students, Fellows, Residents, Interns and Medical Students were not formally registered in 1954 and 1953.

COUNCIL STATEMENT ON PRODUCT ENDORSEMENT

At the 1958-1959 Interim Council Meeting held in St. Louis in January 1959, the Council directed that the following be transmitted to the members of the Association:

The attention of members of the American Diabetes Association is called to the practice of using photographs and citations of individual physicians in the advertising of pharmaceutical products. Members are hereby apprised of the possible misinterpretation thereof by the reader as participation by the individual physician in product promotion or endorsement.

ADA RESEARCH FELLOWSHIP

At least one Fellowship for the academic year 1960-61 (July 1, 1960—June 30, 1961), will be awarded by the Committee on Research and Fellowships of the American Diabetes Association. Deadline for applications is Nov. 15, 1959. Requests for application forms and other inquiries should be addressed to Mr. J. Richard Connelly, Executive Director, who will forward the information to the Committee.

DIABETES WEEK NOVEMBER 15-21

Diabetes Week will be observed the week before Thanksgiving, in accordance with custom. Affiliate Associations and Committees on Diabetes of County and State Medical Societies are urged to plan their Public Education and Detection programs with this date in mind.

A COOKBOOK FOR DIABETICS

A book that is destined to become a best-seller for diabetics will be off the presses by June. It is *A Cookbook for Diabetics,* a 176-page compilation of recipes and menus by Deaconess Maude Behrman, published by the American Diabetes Association and made available at only $1, postage prepaid.

As most everyone knows, Deaconess Behrman is the ADA FORECAST diet expert whose articles have been an invaluable feature of the magazine for over a decade.

Members and readers of the Journal should urge their diabetic patients to reserve their copies now, since the demand for this guide to "good dishes, good meals and good nutritional balance" is building up rapidly.

NEW MEMBERS

The following were elected as of April 1 and May 1, 1959:

Active

Alabama	
Cowart, Norton E.	Huntsville
California	
Cox, J. William	San Diego
Epstein, Marvin A.	Walnut Creek
Colorado	
Arnot, Charles W.	Pueblo
Farrington, John F.	Boulder
Hawaii	
Saunders, Cecil A., Jr.	Honolulu
Illinois	
Anderson, August R.	Harvey
Kloempken, Robert C.	Arlington Heights
Indiana	
Ivy, John H.	Elkhart
Iowa	
Anderson, George S.	Iowa City
Steffey, Frederick L.	Keokuk
Maryland	
Patrick, William H.	Lexington Park
Massachusetts	
Balodimos, Marios C.	Boston
Zeytinoglu, Ibrahim Y.	Boston
Michigan	
Rowe, John B.	Flint
Minnesota	
Kim, Jae Nam	Minneapolis
Mississippi	
Langford, Herbert G.	Jackson
Neill, Karleen C.	Jackson
Witt, William J.	Jackson
Wofford, John D.	Jackson
Missouri	
Mahe, George A.	Clayton
Ramsey, Robert H.	Ferguson
New Jersey	
Tornow, Abraham M.	Jersey City
New York	
Borenstein, Marvin	Westbury, Long Island
Gandy, Hortense M.	Rochester
Heitzman, Edward J.	Syracuse
Poliakoff, Harvey	Rockville Centre
Roberts, Thomas N.	New York
Strachan, John N., Jr.	Niagara Falls
Ohio	
Arquilla, Edward R.	Cleveland
Leonards, Jack R.	Cleveland
Leyrer, Earl C.	Hamilton
Virginia	
Tucker, Henry St. G., Jr.	Richmond
Wyoming	
Holmes, Roy W.	Casper

NEWS OF AFFILIATE ASSOCIATIONS

The CHICAGO DIABETES ASSOCIATION has announced that Charles H. Best, M.D., Director of the Banting and Best Department of Medical Research, Toronto, Canada, will deliver the Woodyatt Memorial Lecture at its third annual "Symposium on Diabetes Mellitus" on Thursday, Oct. 1, 1959. The complete program will be published in a future issue of the Journal.

The FLORIDA DIABETES ASSOCIATION will hold its annual meeting in Miami Beach Oct. 29-30, 1959.

The NEW JERSEY DIABETES ASSOCIATION, in cooperation with the New Jersey State Department of Health, is holding "A Spring Symposium on Neurovascular Complications of Diabetes Mellitus" on Wednesday afternoon, May 13, 1959, at St. Peter's General Hospital in New Brunswick. Chairman of the Session is Gabriel Pickar, M.D. The program includes: Norman Rosenberg, M.D., "Trophic and Ulcerative Lesions of the Lower Extremities in Diabetes"; Robert F. Bradley, M.D., Boston, "Diabetic Triopathy—Retinopathy, Nephropathy and Neuropathy"; Charles Shuman, M.D., Philadelphia, "Diabetic Neuropathy Involving the Autonomic and Parasympathetic Nervous Systems"; and Sherman Gilpin, M.D., Philadelphia, "Central Nervous System Complications of Diabetes Mellitus." A panel discussion on "Neurovascular Complications of Diabetes Mellitus," was moderated by William Levison, M.D., President of the New Jersey Diabetes Association. Panel members included Drs. Rosenberg, Bradley, Shuman and Gilpin.

Calvin E. Oyer, M.D., formerly resident at St. Michael's Hospital in Newark, was presented the Johnson Award at the meeting of the Metabolic Section of the Medical Society of New Jersey on Wednesday, April 29, 1959, for his paper on "The Use of Tolbutamide in Treatment of Diabetes Mellitus: Including a Report of Results in Patients Followed Up to Seventeen Months."

The Clinical Society of the New Jersey Diabetes Association held a regular meeting in East Orange, Jan. 21, 1959. The program included these papers: Herbert Kupperman, M.D., reviewed the newer steroid drugs and their relationship to carbohydrate and electrolyte metabolism; John Gilmore, M.D., in conjunction with William Levison, M.D., reported a case of diabetes in a two-day-old child born of a diabetic mother; George M. Knowles, M.D., related the history of several pregnancies of a juvenile diabetic; and Everett O. Bauman, M.D., reported an unusual case of insulin allergy and resistance.

The TWIN CITIES DIABETES ASSOCIATION (Clinical Society) held a meeting in St. Paul Feb. 24, 1959, as guests of the Ramsey County Medical Association. Howard F. Root, M.D., Boston, spoke on "Cardiovascular Disease in the Diabetic."

The VIRGINIA DIABETES ASSOCIATION is holding its annual program May 7-8, 1959, in Richmond in conjunction with the Ninth Annual Scientific Assembly of the Virginia Academy of General Practice. The Clinical Society will present the following program: "Oral Hypoglycemic Agents," by Leo P. Krall, M.D., Boston; "Eye Complications in the Diabetic," by L. Benjamin Sheppard, M.D.; and "Peripheral Arterial Disease in the Diabetic," by William R. Sandusky, M.D. A panel discussion is being moderated by Thomas S. Edwards, M.D., Charlottesville.

NEWS NOTES

ADA MEMBERS WILL PRESENT POSTGRADUATE COURSE LECTURES

Among those taking part in The American College of Physicians' Postgraduate Course No. 5 entitled "Special Topics in Internal Medicine," to be held at the University of Colorado School of Medicine in Denver June 15-19, 1959, will be: Alexander Marble, M.D., Assistant Clinical Professor of Medicine, Harvard Medical School, Boston, and Dalton Jenkins, M.D., Assistant Professor of Medicine, University of Colorado School of Medicine. Dr. Marble will present "The Place of Oral Hypoglycemic Agents in the Management of Diabetes" and "Prevention and Treatment of Complications of Long-term Diabetes." Dr. Jenkins will moderate the session on endocrine diseases and present papers entitled "Special Considerations in the Diagnosis and Management of Thyrotoxicosis," and "The Challenge of Hyperparathyroidism."

Postgraduate Course No. 6 of the American College of Physicians, entitled "Internal Medicine: Selected Topics," will be held at the University of Cincinnati College of Medicine, in Cincinnati, June 22-26, 1959. Participants will include Harvey C. Knowles, Jr., M.D., Associate Professor of Medicine, University of Cincinnati College of Medicine, who will speak on "Treatment of Diabetic Acidosis," "Oral Hypoglycemic Agents," and "Current Concepts of the Collagen Disorders"; and Emile E. Werk, Jr., M.D., Assistant Clinical Professor of Medicine, University of Cincinnati College of Medicine, who will speak on "The Pharmacological Use of Adrenocortical Hormones." Both Drs. Knowles and Werk will be members of the "Conference on Endocrine and Renal Disorders."

ANNUAL MEETING OF AMERICAN COLLEGE OF PHYSICIANS

Forty-seven members of the American Diabetes Association participated in the Fortieth Annual Session of The American College of Physicians in Chicago April 20-24, 1959.

Among the participants were: Alexander Marble, M.D., who moderated a panel discussion entitled "Questions and Answers in Diabetes" on April 24. Garfield G. Duncan, M.D., Harvey C. Knowles, Jr., M.D., Rachmiel Levine, M.D., and Randall G. Sprague, M.D., were members of the panel.

James F. W. Clark, M.D., Chester Coggeshall, M.D., and Roland P. Mackay, M.D., took part in "Problems in Diabetes" on April 21, as part of the Program of Hospital Clinics.

Robert F. Bradley, Jr., M.D., Leo P. Krall, M.D., A. Macaulay, M.D., J. Pomeranze, M.D., Charles Weller, Jr., M.D., and Priscilla White, M.D., presented a Scientific Exhibit, "A New Oral Hypoglycemic Agent—Phenformin (DBI)."

James B. Field, M.D., delivered a paper entitled "Circulating Insulin Antagonists in Diabetes Mellitus"; and Robert H. Williams, M.D., "Oral Antidiabetic Therapy." James B. Hurd, M.D., was co-author of "Addison's Disease Plus Diabetes."

USC TO HOLD REFRESHER COURSE

The Second Annual Postgraduate Refresher Course of the University of Southern California School of Medicine will be held on board the SS *Lurline* and in Honolulu, July 29—Aug. 15, 1959. Among papers to be presented is "Oral Drugs in the Treatment of Diabetes Mellitus," by John L. Webb, M.D.

PERSONALS

EDWARD L. BORTZ, M.D., Philadelphia, formerly president of the American Medical Association, has been named Chairman of the 1960 National Health Forum sponsored by the National Health Council, to be held in Miami Beach, Florida, the week of March 13, 1960.

JEROME W. CONN, M.D., has been elected to Honorary Fellowship in the American College of Surgeons. His election recognizes Dr. Conn's discovery of primary aldosteronism, a disorder also called "Conn's Syndrome," which climaxed more than ten years of clinical and laboratory observations.

Dr. Conn, Banting Memorial Lecturer of the American Diabetes Association in 1958 and recipient of the Banting Medal, is Professor of Medicine and Director of the Metabolism and Research Unit, University of Michigan Medical School; and Chief, Department of Endocrinology and Metabolism, University Hospital, Ann Arbor.

PIERO P. FOA, M.D., Professor of Physiology and Pharmacology, Chicago Medical School, is participating in a series of seminars at the Universities of Copenhagen, Helsinki, Edinburgh, and Milan, during April and May. Dr. Foa is discussing glucagon, oral hypoglycemic drugs, and other problems of diabetes. He also plans to address the international symposium at the University of Bologne.

NECROLOGY

ISRAEL BECKER, Chicago, Illinois, born Dec. 4, 1899.
JAMES M. BOWERS, Seattle, Washington, born March 28, 1900.
JOHN R. MEADE, St. Paul, Minnesota, born Feb. 28, 1904.